A TEXT-BOOK

OF

PRACTICAL HISTOLOGY

WITH OUTLINE PLATES

BY

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With 30 Outline Plates, 1 Coloured Plate, and 27 Wood Engravings

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TO

CARL LUDWIG

PROFESSOR OF PHYSIOLOGY IN THE UNIVERSITY OF LEIPSIC

AND

L. RANVIER

PROFESSOR OF HISTOLOGY IN THE COLLÉGE DE FRANCE, PARIS

TO WHOM THE AUTHOR IS INDEBTED FOR HIS EARLIEST TRAINING IN THE TRUE METHOD OF MAKING AND RECORDING ORIGINAL OBSERVATIONS IN PHYSIOLOGY AND HISTOLOGY, HE, WITH HEARTFELT PLEASURE, DEDICATES THIS WORK

IN OFFERING TO THEM THIS TRIBUTE OF HIS ADMIRATION AND REGARD, THE AUTHOR DESIRES TO EXPRESS HIS SENSE OF THE INESTIMABLE ADVANTAGE WHICH THEY HAVE CONFERRED ON PHYSIOLOGY AND HISTOLOGY

THEIR LABOURS, CHARACTERISED AS THEY HAVE BEEN BY ENTHUSIASM AND SINGLE-MINDED DEVOTION TO THE ADVANCEMENT OF SCIENCE, HAVE NOT ONLY ADDED GREATLY TO OUR FUND OF KNOWLEDGE, BUT HAVE ALSO DEMONSTRATED THE SUPERIORITY OF THAT METHOD OF SCIENTIFIC OBSERVATION WHICH CONSISTS IN A PATIENT AND INTELLIGENT SCRUTINY OF NATURE

TO THIS METHOD THEY HAVE ATTRACTED MANY DISCIPLES WHO ACKNOWLEDGE THE INCALCULABLE VALUE OF THEIR PERSONAL INFLUENCE, THE CORDIALITY WITH WHICH THEY HAVE ENCOURAGED AND DIRECTED THEM IN THEIR PURSUIT OF ORIGINAL INVESTIGATIONS, AND THE GENEROSITY WITH WHICH THEY HAVE LAID OPEN TO THEM THE RICH STORES OF KNOWLEDGE ACCUMULATED DURING A LIFE-LONG STUDY OF THE PROBLEMS OF ANIMAL STRUCTURE AND FUNCTION

PREFACE.

THE PURPOSE of this work is twofold: first, to give plain, definite, and precise directions for the preparation and examination of the animal tissues, and, secondly, to ensure that the student executes a drawing of the majority of the microscopic specimens which he makes for preservation. For this purpose a series of Outline Plates is issued with the text.

The author has endeavoured to give a faithful account of the methods which he has found to be most useful for the preparation of each of the tissues and organs of the body for microscopic purposes. No method is introduced which he has not found from repeated trials to be successful. The methods described are those which, after nine years' experience in the teaching of practical histology, he has found to be really reliable.

In the introductory chapter a short account is given of the microscope and how to use it, but no attempt is made to explain the optical principles on which it is constructed, as the author believes that such details are better omitted in a work like the present. The reader is therefore referred for such details to any of the numerous works upon that instrument. The student is advised to read the introductory chapter before beginning the practical portion of the work.

The author, from long experience in the teaching of histology, has learnt that the reluctance shown by students in making drawings of their microscopic preparations renders it advisable to facilitate as much as possible this method of exact representation. This he does by giving to each student a series of outline plates in which the main features of the chief microscopic sections are indicated. For two sessions he has done this with his classes of practical histology in the University of Aberdeen, and he finds that the students rapidly acquire the art of filling in the necessary details. At the end of the course of histology, therefore, each student is in possession of a complete set of microscopic preparations, which illustrates the

whole range of normal histology, and which he can permanently preserve and take away with him He has also an atlas representing the details of the more important preparations, and, lastly, in the text-book he has a record of how each specimen was prepared, and a description of the chief points to be observed in each microscopic preparation It is to be remembered, however, that no mere instructions or directions given in books can ever supersede that form of information which is imparted *vivâ voce* by a teacher, and which is elicited by the difficulties that meet the student of histology at the beginning of his course

He is indebted to Mr E A SCHAFER for the use of several of the woodcuts employed in the introductory chapter, and for fig 3, to Messrs Baillière, Tindall, & Cox The outline figures were drawn by the author from the microscopic specimens with which each student is supplied or makes for himself

He has also to express his sincere thanks to Dr ROBERT LAWSON, Deputy Commissioner in Lunacy, and to Dr DE BURGH BIRCH for revising the proof-sheets

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ABBREVIATIONS USED IN THE TEXT

(H) means *high* power, *i e* a magnifying power of 300 diameters linear Such a magnifying power is obtained by using a No 7 objective, and a No 3 eyepiece of Hartnack with the tube drawn out , or an English $\frac{1}{6}$-inch objective and a medium eyepiece

(L) means *low* power, *i e* a magnifying power of 65 diameters linear Such a magnifying power is obtained by using a No 3 objective and a No 3 eyepiece of Hartnack with the tube drawn out , or an English one-inch objective and a medium eyepiece

T S and L S mean transverse and longitudinal section respectively

EXPLANATION

OF

THE OUTLINE PLATES

PLATE I *BLOOD*

(*To face p* 4)

FIG

1 Coloured blood-corpuscle of a newt or frog on the flat
2 Coloured blood-corpuscle of a newt or frog on the edge
3 Effect of acetic acid on a newt's corpuscle
4 Effect of syrup on a newt's corpuscle
5 Effect of tannic acid on a newt's corpuscle
6 Effect of water on a newt's corpuscle
7 Effect of osmic acid and picrocarmine on a newt's corpuscle
8 Colourless blood-corpuscles of a newt or frog
9 Human blood-corpuscles (coloured and colourless) and fibrin
10 Human coloured blood-corpuscle on edge
11 Human coloured blood-corpuscle on the flat, centre in focus and out of focus
12 Human coloured blood-corpuscle crenated
13 Human colourless blood-corpuscles
14 Effect of acetic acid on these corpuscles
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PLATE II *EPITHELIUM*

(*To face p* 12)

1 Squamous epithelium and salivary corpuscles (mouth)
2 Squamous epithelium cuticle of newt (intra-cellular plexus of fibrils)
3 Silvered epithelium (mesentery of a rabbit)
4 Columnar epithelium of the intestine
5 Vertical section of the cornea
6 Chalice or goblet cell from the small intestine
7 Columnar epithelium from a newt's intestine
8 Ciliated epithelium of the trachea
9 The free ends of the cells of fig 8
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11 Transitional epithelium
12 Secretory epithelium

PLATE III *CARTILAGE*

(*To face p* 16)

FIG

1 Hyaline cartilage
2 Transverse section of a rib of a small mammal
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4 Transverse section of a rabbit's or cat's ear (L)
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(*To face p* 24)

1 Fibres of elastic tissue (Lig nuchæ of ox)
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(*To face p* 28)

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FIG

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4 The incisures of Schmidt
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(*To face p* 122)

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2 Milk fresh and after acetic acid (H)
3 Placental villus (L)
4 Placental villus (H)
5 T S of the umbilical cord (L)
6 Part of fig 5 with (H)

DIRECTIONS FOR FILLING IN THE DETAILS IN THE OUTLINE PLATES

As a rule only the outlines of the leading features in the sections are indicated

The outlines are not drawn to any definite scale, but where practicable the figures are of the actual size as seen under a linear magnifying power of three hundred diameters (× 300) or (H), such a relation being noted on one side of the plates devoted to the elementary tissues

The details are to be filled in by the student in pencil (H or HB) and coloured, the latter being done with coloured pencils or water colour

Sufficient dexterity in the manipulation of water colour is, I find, readily acquired by the student, as very few pigments suffice

All the necessary colours are contained in the shilling boxes of moist colour made by Reeves and Sons

A preliminary ground colour or light wash can be laid on in most cases to great advantage

Evenness of colouring is ensured by operating with a full brush upon the paper placed at a gentle slope Commencing at the top of the figure, carry the brush from side to side with a steady even stroke until the whole is coloured Invariably allow one colour to dry before another is applied

It is sometimes convenient to use a dilute solution of picrocarmine as a groundwash, whilst the nuclei and other parts stained red may be tinted with solution of carmine A logwood tint is obtained by mixing a small quantity of carmine with a good blue, such as ultramarine

The leading parts to be filled in are indicated in the text.

ILLUSTRATIONS IN TEXT.

INTRODUCTION.

HISTOLOGICAL REQUISITES

The student of Histology requires the following apparatus —

1 A **compound microscope**, capable of magnifying from fifty to three hundred diameters linear

2 Two short, strong, sharp-pointed **needles**, fixed in wooden handles, at least five inches long, leaving about half an inch of their point projecting (fig 1) Each student ought to make these needles for himself Ordinary sewing needles may be conveniently fixed in crotchet-holders

Fig 1 *n*, End of a Mounted Needle Natural Size

3 A pair of steel **forceps**, which must not be too broad at the points

4 A **hollow ground razor**, for making sections with the hand (p xxxvi) The razor 'made for the army' by John Heifor is the best

5 A pair of sharp-pointed **scissors** It is advantageous for some purposes to have a pair curved on the flat (fig 2)

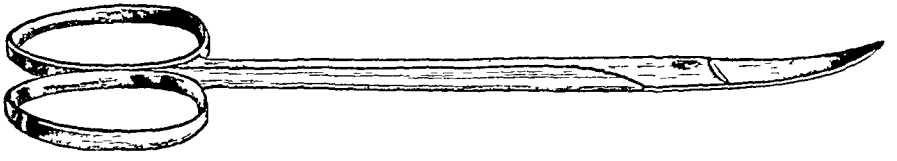


Fig 2 Scissors Curved on the Flat Natural Size

6 Three or four small **camel-hair brushes**—duck size—for putting on the cement used to seal up the preparations It is convenient to keep Nos 2, 3, 4, 5, and 6 in a small box or in a small drawer in one's work-table

7 **Glass slides** —It is desirable to have two sizes The glass should be free from specks and not too thick, and the edges ought to be ground

(*a*) Six dozen ground-glass slides 3 × 1 inch

(*b*) Three „ „ 3 × 1½ „

8 **Cover-glasses** —I prefer the circular form, though many observers use squares The one point of importance about them is that they cannot be too thin It is well to measure their thickness, and this can be easily done as described at p xxv Messrs Chance, of Birmingham, make cover-glasses of three thicknesses, which are numbered Nos 1, 2, and 3

No 1 is the thinnest, and is what ought to be used They vary in thickness from 004 to 008 inch in thickness

(*a*) ½ oz extra thin ¾ inch circles, or No 1
(*b*) ½ oz extra thin 1 inch circles, or No 1
(*c*) ½ oz extra thin 1¼ inch circles, or No 1

These covers ought to be arranged, according to their size and thickness, in a small box divided into compartments by means of small pieces of pasteboard This the student can easily make for himself

9 A **pine-wood box**, or cabinet, provided with trays, so that the specimens may lie on the flat The trays ought to be made both for large and small slides, and the cabinet ought to contain at least six dozen slides Each student in the class of practical physiology mounts over one hundred slides in a session

10 **Reagents**—Each student ought to be provided with a small wooden framework for holding bottles, containing the solutions in common use

It is not of much importance what kind of bottle is employed One-ounce bottles with moderately wide mouths, and fitted with a glass rod passing through the cork, answer admirably The solutions most commonly employed are —

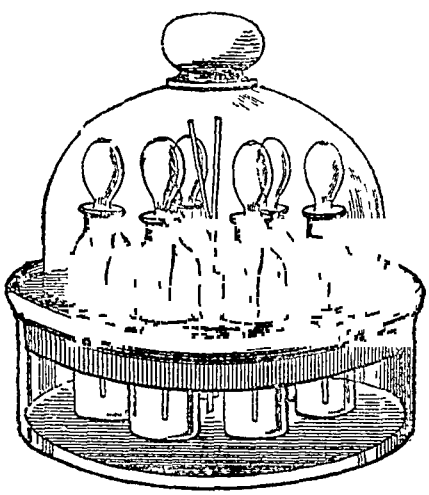


Fig 3 Ranvier's Arrangement for Holding Drop Bottles with Reagents

1 ¾ *per cent salt solution* (p xxx)
2 *Dilute Acetic Acid*, 1 part to 10 of water
3 *Solution of Picrocarmine* (p xliii)
4 *Solution of ordinary Carmine* (p xliii)
5 *Solution of Logwood* (p xlii)
6 *Clove oil*—It is advantageous to use a camel-hair or sable brush fixed to the end of a piece of wood thrust through the cork
7 *Damman Mounting Fluid* (p xlviii)
8 *Glycerine*
9 *Farrant's Solution* (p xlviii)

The other solutions are supplied as they are required

Ranvier uses a very convenient arrangement for holding the reagent bottles (fig 3) It consists of a circular glass vessel, about four inches in diameter and two inches deep Into its mouth a perforated plate of cork is fitted, and in this cork small 'drop bottles' are fixed, and the whole is covered with a glass shade to keep out dust Anyone can easily make such an arrangement for himself

10 Narrow **strips of blotting paper**, which ought to lie in a small tray attached to the stand containing the reagents They are of use to soak up surplus fluid, and also serve as a white background, when such is required, as in teasing certain coloured preparations

Watch-glasses and small *glass capsules* are required, and the student will find the instruments of his *dissecting case* very useful *Glass pipettes* are occasionally required to remove fluid They are easily made by heating a narrow glass tube in a gas flame, and then drawing it out

ON THE USE OF THE MICROSCOPE

GENERAL DIRECTIONS

The microscope used in the Physiological Laboratory of the University of Aberdeen is Hartnack's, model No III A (fig 4) with an eyepiece, or ocular, No 3, and two objectives, or lenses, Nos 3 and 7 The combination of ocular No 3 with objective No 3, gives a magnifying power of about 65 diameters linear, and ocular No 3 and objective No 7 about 300 diameters, with the tube drawn out These powers are quite sufficient for beginners, and, indeed, for most of the ordinary work This microscope costs about £7 Higher magnifying powers are supplied when they are required

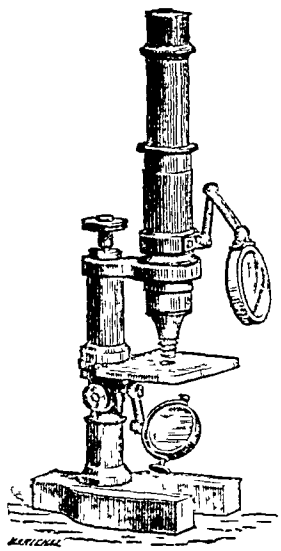


Fig 4 HARTNACK'S MICROSCOPE No III A
One fifth Natural Size The Condenser is attached to the Body of the Microscope

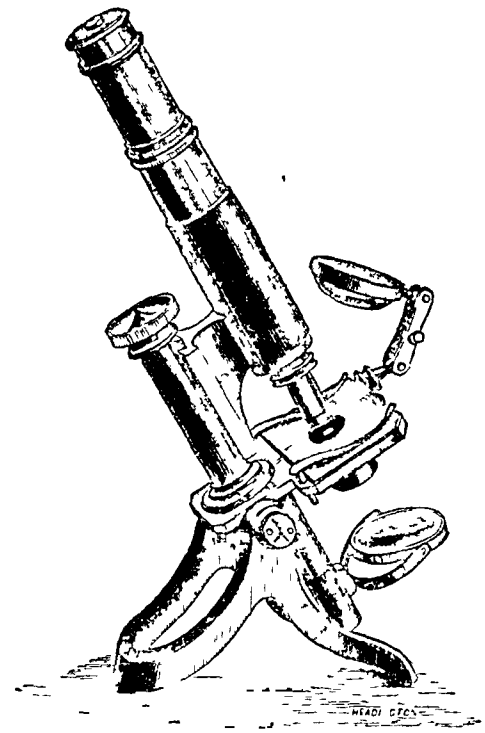


Fig 5 SWIFT'S COLLEGE MICROSCOPE
With a Condenser attached to the Stage for examining opaque objects

The magnifying power can be diminished or increased by pushing or pulling out the draw-tube In fig 4, the draw-tube is represented as drawn out

In Hartnack's microscope, the stand has a horse-shoe form, but many prefer the tripod form (fig 5) as made by Messrs Swift and Son, University Street, London In this microscope the tube is cloth-lined, so that it moves easily, and the stage is covered with a glass plate which enables the slide to move easily on it, and prevents it from being acted on by acids

The *object-glass, objective, or lens*, is the most important part of a microscope, and it is necessary to see that a good lens be procured The lens should be tested by a competent microscopist before it is purchased Coloured blood-corpuscles and salivary corpuscles are

better 'test objects' than the markings of diatoms It is most important to have a good high power such as Hartnack's No 7, equal to about an English $\frac{1}{6}$ inch If a higher power be required, Zeiss' E=$\frac{1}{8}$ inch, and his F=$\frac{1}{12}$ inch, are excellent lenses

IMMERSION LENSES

Ordinary lenses are 'dry,' that is, air is the medium between the object and the objective For higher powers, lenses are specially constructed so that a fluid medium is placed between the object and the objective The effect of this is to collect a larger number of rays of light passing through the object, and so increase the illumination of the field of the microscope Water was formerly employed, and lenses were specially constructed for use with water as the medium Recently, however, *oil immersion* lenses have been constructed by Zeiss of Jena, and his $\frac{1}{12}$ inch is the best lens These lenses are rapidly taking the place of water immersion lenses Similar lenses are made by Messrs Powell and Lealand Cedar oil is the medium employed for these lenses A drop of oil is placed on the lens, and the tube is then lowered till the oil comes in contact with the slide, and the object is focussed in the ordinary way They increase the sharpness and brilliancy of objects immensely As the cedar oil dissolves dammar, all preparations to be examined with oil lenses must be sealed up with marine glue, which is not acted on by cedar oil It is not necessary for the student to get these expensive lenses, they are supplied when they are required

A *nosepiece* is a simple device (fig 6) by means of which the unscrewing of one objective to use another is obviated Either the high or the low power can be used It is necessary that both lenses be accurately centred The nosepiece is screwed to the tube of the microscope, and to it the lenses are screwed A nosepiece may likewise be made to carry three or four lenses

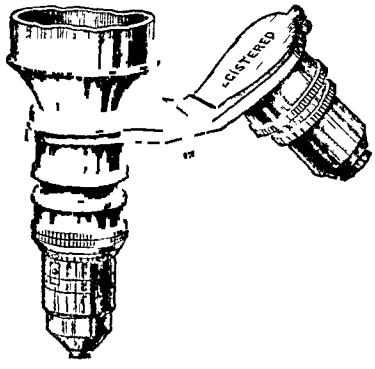


Fig 6 NOSEPIECE MADE BY SWIFT

Eyepieces—Nos 3 and 4 are supplied with Hartnack's microscope but it is well always to use the deep eyepiece, or No 3, *i e* the less powerful of the two

THE ADJUSTMENTS OF A MICROSCOPE

In every microscope there are two adjustments —

(*a*) The *coarse adjustment*, which serves to bring the object roughly within focal distance In Hartnack's microscope, this is accomplished by rotating—not pushing—one tube inside another with the hand This is by far the most convenient method As the tube of this microscope is not cloth-lined, the two surfaces ought to be kept perfectly clean to ensure easy motion If the tube does not move easily enough, rub it with a little almond or watchmaker's oil, and move it upwards and downwards till it glides easily Wipe off the surplus oil with a cloth

(*b*) The *fine adjustment*, is accomplished by rotating a milled head or screw placed at the upper part of the pedestal of the microscope It serves to bring the object accurately into focus after its outline has been brought into view by means of the coarse adjustment

The *stage*, on which the object is placed, ought to be fixed, and at least three inches in breadth In Hartnack's microscope it is placed at a very convenient height above the table, so that when the outer surface of the left hand is resting on the table, the thumb and fore-

finger can be used to move the slide on the stage In this way all unnecessary fatigue of the arm is avoided In Swift's microscope it is glass-plated

The *diaphragm* is placed under the stage, and consists of a circular plate, with a graduated series of round apertures in it When using a high power, turn round the diaphragm until a small aperture—usually the second smallest—is directly under the hole in the centre of the stage A small catch indicates when this is accomplished When using a low power, employ a large aperture of the diaphragm

On the more expensive microscopes small stops, or tubes with graduated apertures, are supplied with the lenses They are slipped into a hole in the stage from below, but care must be taken that they are accurately centred, so that their apertures are exactly under the objective

The *illumination* This is accomplished by means of a mirror placed under the stage Never employ direct sun-light, rather employ light from a white cloud In selecting a site to work with the microscope, try to secure a north light

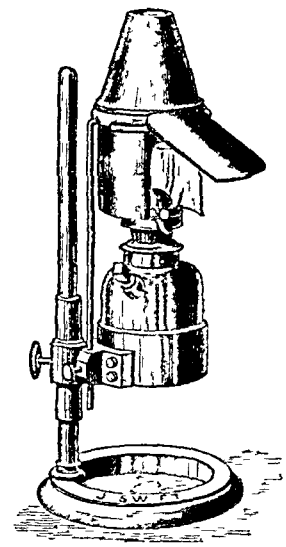


Fig 7 Microscopic Paraffine Lamp (Swift)

ARTIFICIAL ILLUMINATION

Under certain circumstances it is necessary to use a lamp which burns either gas or paraffine I find that an ordinary gas jet, fitted with a Sugg's burner, surrounded by a chimney of pale blue glass, answers admirably Figure 7 shows a form of microscopic lamp which burns paraffine It is also very convenient The light is better when it is transmitted through a light blue glass funnel It is made by Swift

An inexpensive convenient light is obtained from an ordinary paraffine lamp with a flat wick

CLEANING OF THE LENSES OR OBJECTIVES

The lenses are optically the most important part of a microscope Never unscrew the separate parts of the objective or lens If there be any dimness, it is sure to be due to dirt on the outer lenses Carefully rub the lens with a piece of chamois leather, which should be kept ready at hand A convenient way is to tie it to the stand of the microscope If a drop of dammar chance to be on the lens, it must be cautiously removed by applying a drop of clove oil and removing it quickly with chamois leather It is necessary to remove the clove oil at once, as the glasses which compose the lens are soldered together with Canada balsam which would be dissolved by the clove oil A dilute solution of ammonia applied to the lens with chamois leather or cotton wool is an excellent method of removing the greasy film which sometimes coats glass The surface of a freshly-fractured piece of elder pith answers the same purpose admirably

METHOD OF FOCUSSING AN OBJECT

It is extremely desirable for the student of histology to begin at once by learning to keep *both* eyes open when examining an object After putting on the proper lens, arrange the mirror under the stage so as to direct a beam of light up the tube of the microscope and illuminate the field See that the field is brightly illuminated, and that no specks are present.

If specks are present, it may be easily determined as to whether they are on the ocular (eye-piece) or on the objective, if they are on the eyepiece, they will necessarily move when it is moved They must be removed by gentle cleaning with chamois leather

(*a*) LOW POWER (L)—With the No 3 objective Place the tube of the microscope so that the lens is at least an inch and a half above the object Keep looking through the microscope, and, whilst doing so, keep the slide on the stage in motion with the left hand, and rotate or twist the tube downwards—always from right to left—until the outline of the object comes distinctly into view Then employ the fine adjustment, and accurately focus the object

(*b*) HIGH POWER (H)—With the No 7 objective Place the lens a quarter of an inch above the object, and then slowly rotate the tube downwards, keeping the object on the stage in motion all the time with the thumb and fore-finger of the left hand, until the object just comes into view, then employ the fine adjustment As already indicated the magnifying power may be increased by pulling out the draw-tube

Movement of the Slide on the Stage—This is done most conveniently by means of the thumb and fore-finger of the left hand Never employ two hands to move a slide, it is quite unnecessary By practice wonderful precision of movement is acquired Two small clips are placed on the stage, which serve to fix the slide, so that it remains in any position All the cumbrous mechanical appliances formerly in fashion for moving the slide on the stage are quite unnecessary

GENERAL RULES FOR THE EXAMINATION OF AN OBJECT

Always begin the examination of an object with a low power, in order to obtain a general survey of the arrangement of the parts If it be desired to study any particular part, place it directly in the centre of the field of the microscope by means of the low power, and then examine it with a higher power, taking care in the latter instance to use a small aperture of the diaphragm

PREPARATION OF A SIMPLE OBJECT

A drop of blood or milk serves the purpose very well Clean a slide carefully Dip the slide in water, and rub both surfaces at once with a towel which has been washed several times, so as to get rid of all adherent threads It is often convenient for the beginner to use a coloured towel—say, a red one—for if he finds a coloured thread in the field of the microscope, he knows that it has come from the towel Place a small drop of the fluid to be examined on the centre of a slide by means of a clean glass rod Apply a clean cover-glass, taking care to prevent the entrance of air-bubbles

MEHODS OF APPLYING A COVER-GLASS

Seize by the edge a previously cleaned cover-glass with forceps, and allow the drop of fluid to come in contact with the edge of the cover farthest removed from the forceps, gradually and slowly allow the cover-glass to descend obliquely until the fluid forms a uniform stratum under the cover There ought to be just a sufficient amount of fluid to fill out the space between the slide and cover-glass When forceps are not at hand, the cover may be applied with the fingers Take a cover by the margins, rest one edge on the slide close to the drop, allow the fluid to touch the under surface of the edge of the cover, and slowly permit the cover to descend obliquely

METHOD OF CLEANING COVER-GLASSES

Not unfrequently covers are coated with a dull film, which obscures the passage of the light. To remove this film place the cover-glasses for an hour or two in a beaker containing strong sulphuric or nitric acid Pour off the acid, and wash the glasses thoroughly with water until all the acid is removed They may then be placed in methylated spirit, and as each cover-glass is taken out it is cleaned with chamois leather or a well-washed silk handkerchief This is accomplished by rubbing the surfaces of the cover-glass with the handkerchief, held between the thumb and forefinger of the left hand The cover ought always to be lifted by the edges, and never allowed to lie on the flat after it is cleaned, as particles of dirt are apt to adhere to it. Tilt it up against something until required For inch-and-a-quarter cover-glasses a good plan is to have two perfectly smooth pieces of wood, covered with chamois leather, and to place the cover-glass between them, and rub it until cleaned

ON MEASURING COVER GLASSES

As already indicated, No 1 cover-glasses do very well for the ordinary purposes of a student When very high powers are used, it is well to know the thickness of the cover-glass employed Cover-glasses may be rapidly measured in two ways

(*a*) *Micrometer calipers* —This little American instrument (fig 8) is sold by Messrs Chas Churchill and Co, 28, Wilson Street, London It is used for measuring the thickness of fine brass plates The piece in the form of the letter U has a projecting hub, *a*, at one end Through the two ends are tapped holes, in one of which is the adjusting screw, *B*, and in the other the gauge screw, *C* Attached to the screw, *C*, is a thimble, *D*, which fits over the exterior of the hub, *a* The end of this thimble is bevelled, and

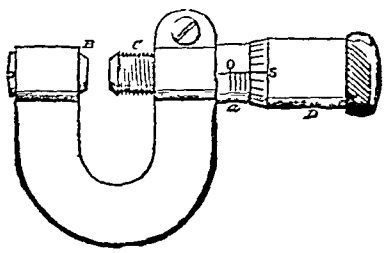


Fig 8 Pocket Sheet metal Gauge for Measuring Cover glasses Natural Size

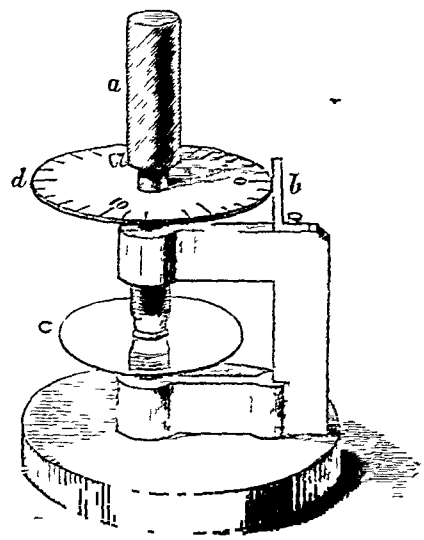


Fig 9 Cover glass Measurer of Zeiss

the bevelled edge graduated into twenty-five parts, and figured 0, 5, 10, 15, 20 A line of graduation, 40 to the inch, is also made upon the outside of the hub, *a*, the line of these divisions running parallel with the centre of the screw, C, whilst the graduations on the thimble are circular The pitch of the screw, C, being 40 to the inch, one revolution of the thimble opens the guage 1-40th or 25-1000ths of an inch. The divisions on the thimble are then read off for any additional part of a revolution of the thimble, and the number of such divisions are added to the turn or turns already made by the thimble, allowing 25-1000 for each graduation on the hub, *a*

Measure an ounce of No 1 cover-glasses, and put aside all those under 004 inch for use with high powers

(*b*) *The cover-glass measurer* (Deckglass-taster) of Zeiss (fig 9) is a very convenient apparatus, which has a movable disc, *d*, divided into degrees The cover-glass, *c*, is placed between the ends of two screws, and the disc is rotated till the screw just holds the cover By an ingenious arrangement of teeth the handle is thrown 'out of gear' when this occurs It gives the thickness of a cover-glass in parts of a millimètre

HOW TO PLACE A SECTION ON A SLIDE

The section ought to be placed in a bowl of water—preferably a white bowl—as it admits of the section being easily seen Take a slide, and plunge it for three-quarters of its length into the water, and with a needle in the right hand gently guide the section on to the slide, which ought to be held in a slanting direction The section is thus easily *floated* on to the slide, and thus all unnecessary tearing of it is avoided The needle is used to hold the section on the slide whilst the latter is being removed from the water Remove the surplus water with a towel To the section on the slide the necessary staining reagents may be applied Sections may easily be treated with alcohol and clove oil on a slide This method is infinitely preferable to lifting sections from clove oil, etc, even with the most approved form of 'section-lifters'

LABELLING OF MICROSCOPIC OBJECTS

This is most important Every preparation, when mounted, ought to be properly labelled Small paper labels are affixed to the slide, and on these are marked the nature of the section, whether it is a transverse (T S) or a longitudinal section (L S), any special points in its structure, how it was prepared and in what medium it is mounted The thickness of the cover-glass in special cases ought to be indicated By using a series of symbols, as Pc for picrocarmine, Lg for logwood, G for glycerine, D for dammar, and F for Farrant's solution, much important information can be put upon one small piece of paper

ON MAKING DRAWINGS OF MICROSCOPIC PREPARATIONS

It is of the utmost importance that each student should make a sketch of many, not necessarily of all, the preparations he mounts This is the only method of ensuring that he sees what is to be seen The outline plates are designed to aid the student in this task Once the student has made a drawing of the details of a preparation he is not likely to forget them It is far more important that each student should thoroughly understand a selected series of preparations than that he should carry off with him a couple of hundred slides, few of which he understands

Various instruments are used for making drawings of microscopic preparations, but it is to be remembered that they are in many cases more useful for giving the actual size of an object as seen with a certain combination of lenses, and for giving the general outline of a section, than for the individual details

Camera Lucida of Chevalier—This instrument (fig 10) is made by Hartnack Instead of the eyepiece, the tube s' is fixed in the tube of the microscope The observer looks through the small prism, and sees the outline of the section thrown on the table, or on a piece of paper laid on the table, close to the microscope A shade is placed in front of this, and must be so adjusted that too much light does not fall on the paper The contour is then mapped out with a pencil

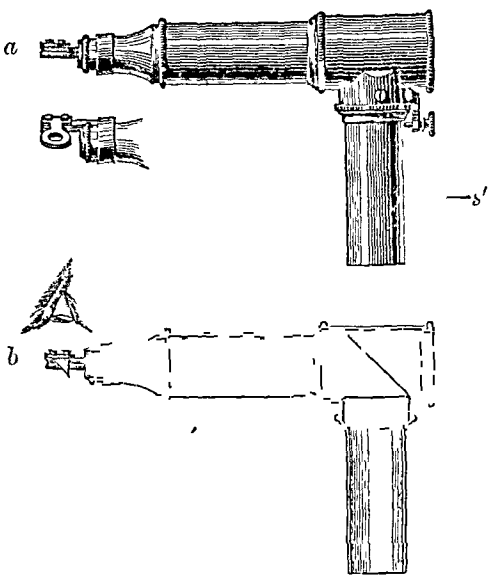


Fig 10 Camera Lucida of Chevalier
a the Instrument, *b* a section, showing the prisms

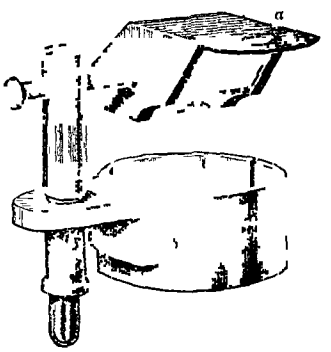


Fig 11 Camera Lucida (Zeiss)
The ring of brass fixes it to the tube of the Microscope, and the aperture *a* is placed over the Eyepiece

Other Cameras—Much simpler forms are the camera lucida of Nachet, or the one made by Zeiss (fig 11) The ocular is left in its place, and the camera is fixed to the tube of the microscope by the ring of brass The aperture *a* is placed immediately over the centre of the eyepiece On looking through this aperture the surface of the table in front of the microscope is seen at the same time as the object The drawing-paper is placed on an *inclined plane* on this part of the table, and the outline of the object is sketched, whilst the details are afterwards filled in without the camera

Neutral tint reflector—This simply consists of a small cap, which fits on the end of the microscope, and in it is placed a piece of glass, at an angle of 45° The microscope tube must be horizontal On looking through the glass the image of the object is seen on the paper The same precautions with regard to the modification of the light on the paper must be taken as for Chevalier's camera

It is not even necessary to have a brass cap to fit on to the microscope, an ordinary cover-glass, fixed to the eyepiece, by means of modeller's wax, at an angle of 45°, answers the purpose admirably

METHODS OF STUDYING THE PROPERTIES OF LIVING PROTOPLASM

THE MOIST CHAMBER

Moist chamber of v Recklinghausen—This is easily made by cutting off the lower end of an ordinary glass cylinder, such as is used for a paraffine lamp Through the lower part of the cylinder the tube of the microscope is placed The lower end of the cylinder is ground flat,

and rests on a plate of glass, to which it is fitted air-tight with oil The inner surface of the cylinder is partly coated with filter paper moistened with water, so that the air in the enclosed space is always kept saturated with watery vapour To close the space all that is required is to tie a layer or two of sheet caoutchouc over the lower end of the tube of the microscope, and the upper end of the glass cylinder The object to be examined is placed on the floor of this chamber, and may be examined either with or without a cover-glass

Simple moist chamber —A very simple chamber may be made by fixing a ring of glass, a quarter of an inch in height and seven-eighths of an inch in diameter, to an ordinary slide by means of dammar solution A drop of water is placed in the chamber, which is then covered with a cover-glass, on which the fluid to be examined is placed The cover-glass is so applied that the drop of fluid hangs into the chamber, as is shown in fig 14

METHODS OF APPLYING HEAT TO MICROSCOPIC OBJECTS

Various forms of warm stages are in use , Max Schultze was the first to use them

Simple form of warm stage —This consists of a thin sheet of copper, three inches long and one and a half inches broad (fig 12) In the centre is a hole half an inch in diameter From one side of the plate a narrow copper arm, five inches in length, projects This is placed on the stage of the microscope, and on it is placed the preparation to be observed If it be blood (p 4), the cover-glass must be sealed round with oil to prevent evaporation, and the whole is fixed by the clips on the microscope Heat is applied to the brass arm by means of a

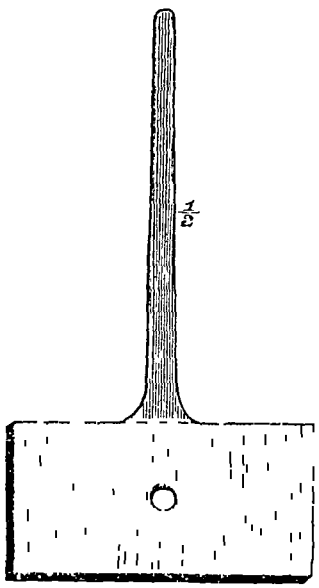


Fig 12 Simple Warm Stage
Half Natural Size

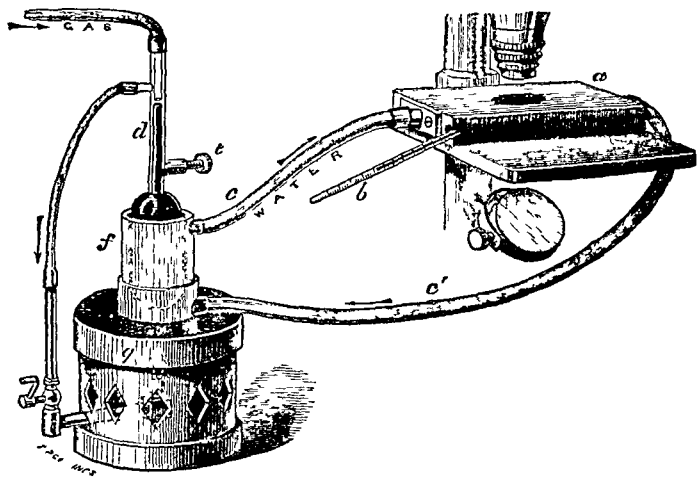


Fig 13 Warm Stage and Apparatus for Maintaining a Constant Temperature under the Microscope (Schafer)

spirit-lamp, and, in order that it may not be over-heated, a piece of a mixture of cocoa-butter and white wax is placed on the copper plate near the preparation This mixture is so made as to melt at a temperature of 30° C When it melts the source of the heat must be removed This does very well for the purposes of the student , anyone can make it for himself from sheet copper For accurate experiments it is necessary to have a thermometer, so that the degree of heat can be accurately measured Such an arrangement is found in Stricker's warm stage

Mr Schafer's constant temperature apparatus—The following apparatus, devised by Mr Schafer, is an excellent one (fig 13) It consists of a brass box, *a*, just like a Stricker's warm stage, having a hole in its centre to admit light to the microscope In it is a thermometer, *b* This box lies on the stage of the microscope It is connected by elastic tubes with a hollow jacket, *f*, and the whole system is filled with water previously boiled to expel the air The water is warmed at *g* by a small gas-flame, the size of which is regulated by a modified Bunsen's regulator, *d* By means of the screw *e* the temperature is regulated at starting

METHOD OF APPLYING VAPOURS AND GASES

A simple apparatus is described at p 11 for applying the *vapour* of *ether* or *chloroform* to an object, *e g* to cilia It is made in the same way as the moist chamber described at p xxviii

If *gases*, such as oxygen or carbonic acid, are to be applied, two tubes require to open

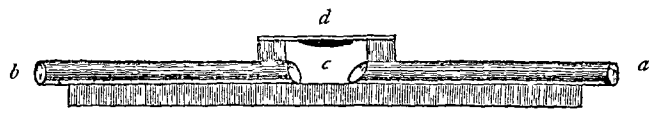


Fig 14 Section of a Gas Chamber *a*, Entrance, *b*, Exit tube, *c*, Object, *d* Cover glass Natural size

into the glass cell, one for the entrance of the gas, and the other for its exit Fig 14 shows a section of such an arrangement The cover-glass, with the object on it is inverted over the cell Stricker's warm stage is usually so made that it can be used for this purpose

ON THE PREPARATION OF TISSUES FOR MICROSCOPIC EXAMINATION

FRESH TISSUES

FRESH tissues as a rule are unsuited for examination with the microscope, though under certain circumstances it is advisable to examine a fresh tissue When this is done, the tissue must be examined in a fluid which will alter its characters as little as possible, such fluids are called

NORMAL OR INDIFFERENT FLUIDS

They closely resemble in composition some of the fluids in which the tissues of the body are bathed

1 **Aqueous humour of the eye** —This is easily obtained by puncturing the cornea of an eye-ball removed from an ox newly killed

2 **Blood-serum** —Pour blood into a tall vessel, and allow it to coagulate After the blood coagulates, run a knife between the upper margin of the clot and the vessel, to permit the clot to contract and sink in the serum, which will be squeezed out of the clot After twenty-four hours draw off the yellow-coloured serum with a pipette

3 **Iodised serum** —Add iodine to blood-serum, prepared as above, until the fluid is of a distinctly yellow colour This fluid alters the tissues slightly, however, and colours them yellow A similar solution may be made by adding iodine to amniotic fluid

4 **Salt solution** (three-quarter per cent)—Dissolve 7 5 grammes sodic chloride in 1,000 c c of distilled water This is by far the most convenient fluid to employ Its composition is so near that of lymph—the fluid normally bathing the tissues—that it alters fresh tissues very slightly

HOW TO HARDEN TISSUES

GENERAL DIRECTIONS

Tissues, however, generally require to be hardened before they can be cut into sections for examination This is accomplished by exposing the tissue to the action of certain fluids

The Material to be employed —The tissues should in all cases be as fresh as possible When possible the tissues ought to be obtained from the human body, though these in all cases may not be available It is therefore necessary to use the corresponding organs of some of the lower animals, *e g*, cat, dog, rabbit, or pig Practically their structure is identical with

that of man The tissue to be hardened ought to be cut into small pieces—half to one inch square—by means of a sharp razor If foreign matters are to be removed from the tissue—*e g* the contents of the gut—do not wash them with water, but with a stream of normal salt solution (p xxx) Place the tissues in a relatively large quantity of the hardening fluid, at least twenty times the bulk of the tissue Change the fluid at the end of the first, third, seventh, and tenth days It is better to place the tissues in a shallow but wide bottle See that the tissues do not stick to the wall of the vessel, else the hardening fluid is prevented from having access to them Cotton wool moistened with dilute alcohol answers admirably as a bed for the tissues to rest on and to separate one piece from another Place the bottle in a *cool place* The hardening will be effected in from one to four weeks At the end of this time remove the tissues, wash them thoroughly in water, or let them steep in a large volume of water for several hours to get rid of the hardening reagent, as in some cases—*e g* chromic acid—its presence interferes with the process of staining Place the tissues in equal parts of methylated spirit and water for two days, and then transfer them to methylated spirit to complete the hardening If the spirit becomes cloudy it must be changed This keeps the preparations until they are required for sections Instead of merely placing the tissue in the hardening fluid, in many cases it is advisable to *suspend it by means of a thin cord in the fluid*, which permits the fluid to penetrate the tissue more rapidly

The process of changing the various hardening fluids on certain days appears tedious, but it is not so in reality, if the tissues to be hardened are placed on a shelf by themselves Chromic acid preparations require to have the fluid changed more frequently than when Muller's fluid is employed For convenience, therefore, they may be placed by themselves On all occasions *label each bottle*, and note the tissue, from what animal it is taken, the name and strength of the hardening fluid, and the date on which it was changed Attention to these apparently small details is the necessary condition for successful histological work

HARDENING SOLUTIONS

The following are the solutions in most common use

A THOSE WHICH HARDEN TISSUES WITHOUT COLOURING THEM

1 **Chromic acid 1 per cent solution** —Dissolve 10 grms chromic acid in 1,000 c c water So strong a solution is not used, as it renders the tissues too brittle It is convenient to keep a strong solution, which can be diluted as required A $\frac{1}{4}$ or $\frac{1}{6}$ per cent solution is generally used

2 **Chromic Acid and Spirit Solution** —This is one of the most useful solutions Make a $\frac{1}{6}$ per cent solution of chromic acid, either by diluting the above, or dissolving one gramme of chromic acid in 600 c c of water Take one part of this, and add two parts of methylated spirit It is better to prepare this mixture as it is required As heat is developed by the mixing, the tissues must not be put into the mixture till it becomes cool It hardens in from seven to ten days, according to the size and nature of the tissue

3 **Potassic Bichromate Solution** —Dissolve 20 grms in 1,000 c c water The solution is most easily made with warm water, but it must be allowed to cool before it is used If the solution is changed every four days it hardens tissues in three to four weeks, but usually a longer time is required, as it is not necessary to change the fluid so often

4. **Ammonium Bichromate Solution**—Dissolve 20 grms in 1,000 c c water This is very useful for hardening the brain and spinal cord, and nervous structures generally

5 **Ammonium Chromate Solution**—Dissolve 50 grms of ammonium chromate in 1,000 c c water, *i e* a five per cent solution, or it may be made by adding one ounce of the salt to twenty fluid ounces of water Filter and preserve in a stoppered bottle It hardens fresh tissue, such as the mesentery, in twenty-four hours After hardening, the tissue must be washed until no more colour is removed by the water It may then be stained and mounted in glycerine For other cases, the kidney for instance, small pieces are hardened for forty-eight hours, and after being thoroughly washed are then treated with spirit, as directed above (p xxxi) This substance is of the utmost value for revealing the rod-like structure in the renal epithelium, and for demonstrating the existence of the intra-cellular and intra-nuclear plexus of fibrils in cells

6 **Muller's Fluid**—Dissolve 25 grms potassic bichromate and 10 grms sodic sulphate in 1,000 c c water This is a most useful reagent, as it penetrates more readily than chromic acid, though it takes longer time to harden tissues—usually from five to seven weeks

It is frequently advantageous to combine the use of two of the above fluids, thus place the tissue for a week in Muller's fluid, and complete the hardening in No 2 After hardening, the tissues are well washed, and preserved in spirit

7 **Muller's Fluid and Spirit**—Mix 3 parts of Muller's fluid and 1 part of methylated spirit This does very well for nerve-tissues, muscle, and the retina It ought to be prepared fresh when required, and the tissue ought not to be placed in it till the mixture cools after the addition of the spirit. To prevent the separation of the chromium salts, which is apt to occur, keep the mixture in a dark place

8 **Methylated Spirit** is sometimes, though rarely, used alone

9 **Absolute Alcohol of S G 0 795**—This hardens very rapidly—in twenty-four hours—but it causes considerable shrinking, though it is invaluable for gastric mucous membrane, and for secretory glands generally, *e g* the salivary glands and pancreas Tissues become stained very readily after hardening in pure alcohol

B THOSE WHICH HARDEN, AND AT THE SAME TIME COLOUR THE TISSUE

10 **Picric Acid**—Make a watery solution saturated in the cold Keep crystals in the bottle to ensure saturation Tissues ought to be left in this only for a day or two, else they are rendered too brittle Its stain is of a bright yellow colour, which is easily removed by prolonged washing in water (Ranvier) Tissues hardened in this fluid become easily stained with picrocarmine

11 **Kleinenberg's Picric Acid** is a modification of the above To 100 c c of a cold saturated watery solution of picric acid add 2 c c of strong sulphuric acid, which throws down a yellow precipitate Filter, and to the filtrate add 300 c c of distilled water This solution is most valuable for fœtal tissues, and especially for early embryos It produces its effects in from three to ten hours

12 **Osmic Acid.**—A one per cent watery solution is most useful, which can be diluted as required A $\frac{1}{4}$ per cent solution, prepared with distilled water, is usually employed It decomposes very rapidly when in contact with organic matter and exposed to light, and as it is very volatile, it must be kept in a glass-stoppered bottle, covered with paper, to protect it from the light It hardens in from six to twenty-four hours, and acts specially on fatty matters, which it blackens, and is very useful for tracing the course of medullated nerve-fibres,

as it blackens the myeline Small pieces of tissue exposed to the vapour of a one per cent solution for a few minutes, are easily stained with picrocarmine, though the prolonged action of a strong solution renders subsequent staining difficult It is one of the most useful of all hardening reagents (M Schultze) It is not necessary to mount preparations treated with osmic acid in a saturated solution of acetate of potash.

METHODS OF SOFTENING TISSUES

Certain tissues, as bone and tooth, which contain calcareous matter, require to have the calcareous matter removed before they can be conveniently cut for the microscope This is done by placing them in a decalcifying solution Some solutions remove only the calcareous matter —*e g* dilute hydrochloric acid—without at the same time hardening the tissue, others, as a mixture of chromic acid and nitric acid, remove the lime salts, and at the same time harden the tissue, and generally the latter are to be preferred

DECALCIFYING SOLUTIONS

1 **Hydrochloric Acid**—Mix one part of strong acid with ten parts of water This does very well for the removal of lime salts from an injected bone

2 **Chromic and Nitric Acid Fluid**—Make a ½ per cent solution of chromic acid, and to every 100 c c add 1 c c of strong nitric acid The chromic acid solution is easily made by adding an equal volume of water to a one per cent solution (p xxxi) This fluid takes two or three weeks to remove the lime-salts from a small piece of bone, but if the fluid is frequently changed the result is accomplished more rapidly The tissue is then thoroughly washed to get rid of the acids, and then hardened, first in weak and then in strong methylated spirit The advantage of this fluid is that the chromic acid hardens the parts, whilst the nitric acid removes the lime-salts Bones, after being exposed to its action, assume a green colour, owing to the formation of a sesquioxide of chromium

3 **Picric Acid Solution**—A saturated watery solution takes several weeks to act on a moderately large piece of bone It is of great value for fœtal bones

4. **A 10 per cent Solution of Common Salt and Hydrochloric Acid**—This is most valuable for showing the matrix of bone, which consists of ordinary fibrous tissue, and swells up in the ordinary acid media A 10 per cent solution of salt prevents this (v Ebner and De B Birch) The bone is placed in a 10 per cent solution of common salt, to which 1 to 3 per cent of hydrochloric acid is added Add from day to day as much acid as will decalcify the bone When the bone becomes flexible it is placed for several hours in water, to remove all the acid Leave it for several days in 10 per cent salt solution, which must be changed repeatedly When the reaction of the bone becomes neutral the bone is white and opaque Sections are made, and mounted in a 10 per cent solution of salt. They show the fibrillar structure of the matrix (p 32) (v Ebner)

METHODS FOR DISSOCIATING TISSUES.

Various solutions dissolve or soften certain parts of a tissue whilst other parts are left unaffected The result is that the component parts may be readily separated by teasing The piece of tissue ought not to be larger than a pea The result is usually effected in from twenty-four to thirty-six hours, though much less time may suffice

DISSOCIATING SOLUTIONS

1 **Iodised Serum** —Add iodine to blood-serum or amniotic fluid, till the fluid is of a distinctly yellow colour This fluid dissolves the cement-substance between cells in from one to two days

2 **Dilute Chromic Acid** (01 *per cent*) —Dissolve 1 grm chromic acid in 10,000 c c water, or dilute a one per cent solution This does excellently for isolating the fibrillæ of muscle, and for the nerve-cells of the spinal cord Two to three days' maceration serves to bring about the result

3 **Dilute Alcohol** ('Alcool au tiers') —Mix 2 parts of water with 1 of rectified spirit This is one of the most useful dissociating fluids, and requires one to two days for its action (Ranvier)

4. **Saturated Aqueous Solution of Baric Hydrate** requires about twenty-four hours to act on the fibrillæ of tendon

5 **Caustic Potash** —Dissolve 40 grms of caustic potash in 100 c c water This isolates muscle-cells in from twenty to thirty minutes

6 **Ten per cent Solution of Common Salt** is useful for dissolving the cement of white fibrous tissue It takes several days to act It is very useful also for showing the fibrillæ of the matrix of bone

7 **Nitric Acid and Glycerine** —Mix one part of strong nitric acid containing nitrous acid with 3 parts of water and 1 part of glycerine The object is placed in this mixture for two or three days and then in water It is specially useful for isolating nerve-structures and lens-fibres (Freud)

DIGESTION AS A HISTOLOGICAL METHOD

This method was introduced into histology by the author several years ago It has recently been employed by Kuhne for investigating the structure of nerves, and by De Burgh Birch in his investigations on the composition of the matrix of bone (p 32) Either an artificial gastric or pancreatic juice may be employed

Artificial Gastric Digestion —This method is fully described at p 92 (W Stirling)

Artificial pancreatic, *i e* trypsin, Digestion —Either an aqueous or glycerine extract of the pancreas may be used The latter is the more convenient It is made thus by v Wittich's method The pancreas of a dog is chopped up and is dehydrated with absolute alcohol for twenty-four hours The alcohol is removed and sufficient pure glycerine is added to cover the gland, and it is allowed to stand for three weeks Press the glycerine through muslin to remove the gland tissue The glycerine is a solvent for the *trypsin* of the pancreas, just as it is

for other soluble ferments 1 c c of the glycerine filtrate is added to 19 c c of 1 per cent solution of sodic carbonate The fluid becomes turbid, but after filtration a pale yellow fluid is obtained The tissue to be digested is placed in this fluid, and the whole is kept at a temperature of 40° C in a water bath as directed at p 92 Sections of softened bone digested by this method are preserved in a 10 per cent solution of common salt (Birch)

METHODS OF TEASING A TISSUE

Take only a *small* piece of tissue, and place it on a slide in a small drop of the fluid in which it is to be mounted, *e g* glycerine Fix one end of the tissue with a strong needle in a handle, and tear in the direction of the fibres with the other needle The low power must be used from time to time, to ascertain when the component parts are sufficiently isolated It is very desirable to have a proper background, so that the object to be teased may be distinctly seen , for a coloured object a small piece of white bibulous paper answers admirably, and a transparent object is generally best seen on a dark surface A good plan is to have a narrow white line painted along the edge of the work-table The same result is obtained by having a small slab, one-half of which is glazed white and the other black

RANVIER'S PHOTOPHORE

The following little arrangement devised by Ranvier is of great value for this purpose It is called a *photophore* (fig 15) It consists of a small mahogany box about four inches square , one side is open and so is the top The lid is formed of a glass plate Into the box a mirror is fixed at an angle of 30°–35° This arrangement reflects the light through the preparation If it be desired to work with a black background, all that is required is to place a black card over the mirror This simple arrangement is of the utmost value, and when in use has simply to be directed with its mirror towards a window It is well to have a bent piece of wire, with a ring formed on it and attached to the back of the box, for receiving a small magnifying lens Each student should be provided with such an arrangement He can easily make one for himself by taking an ordinary small cigar-box without a lid, and placing in it a mirror at an angle of 30°–35° On the upper side make a square hole, and into this fit a glass plate

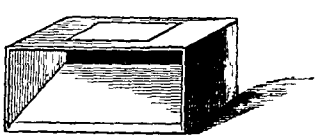


Fig 15 RANVIER'S PHOTOPHORE
One sixth Natural Size

SIMPLE OR DISSECTING MICROSCOPE

In teasing or dissociating a tissue it is often of great value to use an ordinary dissecting microscope The form devised by Brucke and made by Hartnack is useful, but it is expensive For practical purposes I find that the form, fig 16, made by Swift, is a most useful and handy instrument

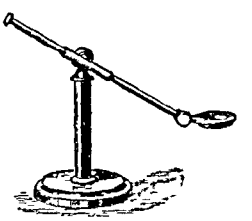


Fig 16 SWIFT'S SIMPLE DISSECTING MICROSCOPE
One sixth Natural Size

METHODS OF CUTTING SECTIONS

It is of the utmost importance that the student should thoroughly master the details of cutting sections by hand, and also with the aid of certain instruments called *microtomes*

I SECTIONS OF UNHARDENED TISSUES

If it be desired to examine only a small piece, snip off a thin fragment with a pair of scissors curved on the flat, or cut off a slice with a Valentine's knife

II SECTIONS OF HARDENED TISSUES

1 **Cutting sections by hand with a razor**—Tissues hardened by any of the above-mentioned methods are difficult to cut unless they are clamped or imbedded in some easily cut material, which must have nearly the same consistence as the tissue itself As to the kind of razor or knife to employ, the 'army razor' answers admirably, though some prefer specially constructed knives, which, I believe, possess no advantages, whilst others prefer the razor to be ground flat on the side directed towards the tissue to be cut

If the piece of tissue be sufficiently large, seize it between the thumb and fore-finger of the left hand Hold the fore-finger horizontally, so that its outer surface forms a rest on which the blade of the razor may glide Keep the handle of the razor in a line with the blade, and hold it firmly in the right hand *Draw* it (from heel to tip) through the tissue towards yourself *Keep the razor well wetted with spirit*, and float the section off into spirit or water, with a camel-hair pencil It requires very considerable practice before one becomes expert at making sections of sufficient thinness to be of use The razor must be kept perfectly sharp, and after making a few sections should always be 'stropped'

If the tissue be too small or too delicate to hold in the hand, it must either be clamped or imbedded in some substance

(*a*) A piece of *amyloid* or *waxy liver*, or the liver of a pig hardened in alcohol, serves the purpose admirably Make a slit in the piece of liver, and clamp the tissue in it, and cut both liver and tissue as directed above

(*b*) *Carrot* or *elder-pith* may be used instead Make a slit in the carrot or pith, and clamp in it the tissue to be cut.

IMBEDDING MIXTURES

(*a*) Melt together with the aid of a gentle heat four parts of ordinary solid paraffine with one of pure lard A quantity of this mixture should be kept ready for use When required, the mixture is to be dissolved in a vessel heated in a water-bath at the lowest temperature possible.

Method of Imbedding—Press the tissue to be imbedded between folds of blotting-paper, to remove all the alcohol, and see that its surface is thoroughly dry It is a good plan to dip it for a moment into a weak solution of gum arabic, and allow the gum to dry as a film on its surface This prevents the paraffin from penetrating the tissue, and also causes it to separate more readily from the section Fold a piece of paper in the form of an oblong box, and pour into it a little of the previously melted paraffine Place a needle into the tissue, fix it conveniently in the paraffine mixture in the paper box, add more paraffine until the tissue is well covered Use the needle to keep the tissue in its place until the mixture sets The paraffine block is then removed from the paper, and sections cut through it and the imbedded tissue The sections are floated off into spirit or water

(*b*) Other imbedding mixtures are sometimes used, as two parts of paraffine mixed with one of vaseline, which gives a transparent mass, in which the position of the tissue can be detected

MICROTOMES

When a large number of sections is required, or when a complete section is wanted through an organ, there can be no doubt that a *microtome* of some kind is required One of the earliest microtomes introduced (fig 17) is that of Mr A B Stirling, of the Anatomical Museum, Edinburgh University It consists of a strong brass tube, with a broad, smooth, metal plate fixed at right angles to it at one end In this cylinder a plug is moved by a very fine-threaded screw, which works in the lower end of the tube The instrument is so made that it can be clamped to a table The tissue to be cut is imbedded in the well of the microtome in paraffine and lard, in the same way as a tissue is imbedded for cutting hand sections Melt the imbedding mixture, and fill the well of the microtome, and whilst still fluid put in the tissue to be cut—using the same precautions as to drying—and hold it in position till the mixture sets By turning the screw the mass and included tissue are elevated , and a sharp knife carried along on the flat brass plate shaves off a section, which can be made as thin as desired by having a screw of sufficient fineness The knife must be kept flooded with spirit, and the sections are floated off by means of a camel-hair brush into spirit or water

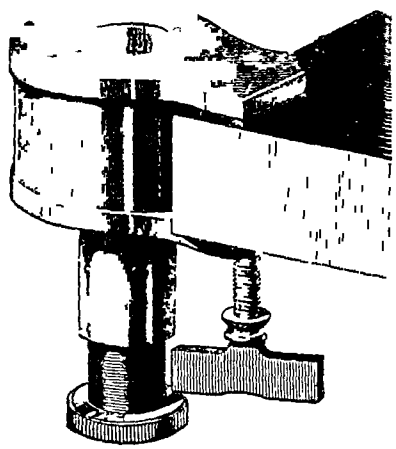


Fig 17 Mr A B Stirling's Microtome

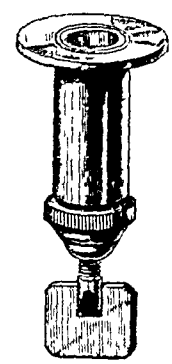


Fig 18 Ranvier's Hand Micpotome
Half Natural Size

Ranvier's Microtome is simply a smaller instrument, working on the same principle, and designed to be held in the hand (fig 18) The whole apparatus can be inverted in spirit, if it be desired to make sections of the same tissue from time to time

Sections to be cut in Ranvier's microtome are imbedded just as in Mr Stirling's form, with a paraffine and lard or other mixture Elder-pith, however, may be used The tissue is packed in with *dry* elder-pith, and then the whole apparatus is placed in spirit, which causes the pith to swell, and so to fix the tissue sufficiently firmly for a section to be made of it

FREEZING MICROTOMES

Dr Rutherford's Freezing Microtome—This microtome (fig 19) is essentially a modification of Mr Stirling's microtome The hollow brass cylinder is surrounded with a large trough (G), which is filled with a freezing mixture composed of equal parts of salt and ice The trough is provided with a tube (H) to permit of the outflow of the water resulting from the melting of the ice It is a great improvement to have the cutting-table covered with a thick plate of glass, as this is not worn away so readily as the brass This microtome can be used as an ordinary microtome, as well as for freezing

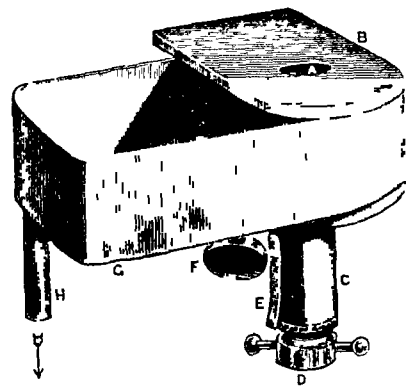


Fig 19 Dr Rutherford's Freezing Microtome

B, Plate of brass with aperture A leading into the well of the Microtome, C Lower end of cylinder in which the screw D works, moving a brass plug up or down, G, Trough to hold freezing mixture, H, Ex t tube for water, F, Clamp to fix the instrument to a table

METHOD OF USING A FREEZING MICROTOME

Preparation of the Microtome—Clamp the instrument to a table Turn the screw (D) until the plug has descended a good distance into the well, and to prevent the screw freezing pour a little alcohol into the well and let it run through the tube, dry the well thoroughly, and smear a little lard round the top of the plug, to prevent the escape of any of the imbedding mixture between the plug and the wall of the cylinder, which would interfere with the action of the screw

Preparation of the Tissue to be Frozen—The tissue has first been properly hardened by one of the foregoing methods If it has been kept in alcohol, it must be placed in a large quantity of water—to be changed several times—during twenty-four hours, to remove all the alcohol It is then placed for twenty-four hours in gum-mucilage (B P) The well is then filled with gum (B P), and equal quantities of powdered ice and salt are packed into the trough with a piece of stick until the edge of the gum is observed to freeze, when the piece of tissue is placed in the gum in the side of the well nearest the side from which the person makes the sections, by means of a needle The freezing process is continued until the whole mass of gum becomes solid, the gum becoming of such a consistence that it cuts like a piece of cheese (U Pritchard)

The Section Knife—The ordinary razor does not answer well for cutting sections with the microtome Its blade is too thin and too short It is necessary to have a stout blade, seven or eight inches long, with a *thick straight back*—at least a quarter of an inch thick—fixed in a straight handle The blade must be so strong that it cannot be bent whilst pushing it along the glass plate The surface next the glass plate ought to be slightly concave The surface of the knife ought to be moistened with gum solution, which prevents the sections from curling up In cutting, keep the back of the knife directed towards the operator, and *push* the blade *obliquely* from tip to heel, guiding it with the thumb and forefinger of the left hand through the tissue Each section as it is made must be floated off into a bowl of water with the aid of a large camel-hair brush (See p xl for how to preserve them)

MODIFICATIONS OF THE ABOVE METHOD

In the preparation of the Tissues (*a*)—For some tissues—*e g* brain—it is necessary to leave them forty-eight hours in the gum before cutting

(*b*) The following method is an excellent one, and is of the greatest value for very delicate tissues as the retina, ear, brain After soaking the tissues in water, place them for twenty-four hours in syrup made by dissolving four ounces of crystallised sugar in two ounces of water They are afterwards placed in gum for twenty-four hours, and are then ready for cutting

With such a microtome as described above there is no difficulty whatsoever in making the most perfect sections The well can be made of any size, and, indeed, D J Hamilton, of Edinburgh, has had a large freezing microtome constructed which enables him to make microscopic sections of an entire brain

WILLIAMS' FREEZING MICROTOME

Some histologists prefer this form of microtome (fig 20) It is made by Swift, of London, and consists of a wooden tub for the freezing mixture In this is an upright, brass bar, into whose upper end the circular brass plates, on which the tissue is frozen, are screwed A glass top with a hole in its centre, through which the circular plate projects, forms a lid for the box The knife is fixed in a triangular frame, provided with screws by which it can be raised or lowered

METHOD OF USING WILLIAMS' MICROTOME

Remove the lid of the box and fill the chamber with equal parts of pulverised ice and salt, care being taken not to allow the mixture to touch the under side of the cover, which, when

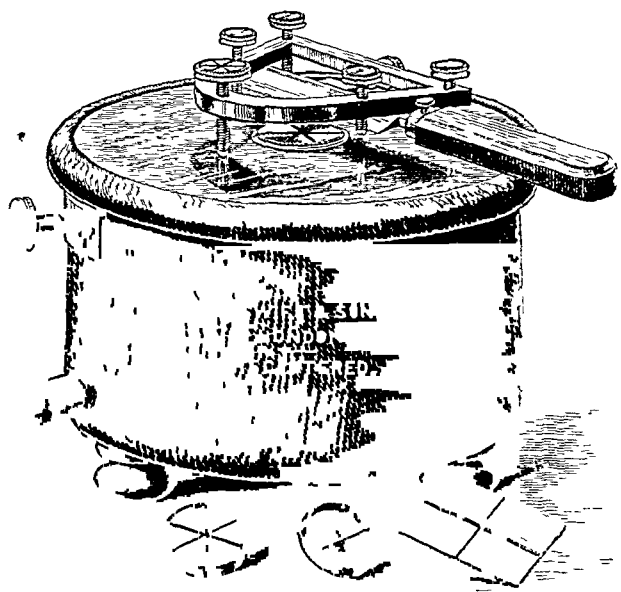


Fig 20 Williams' (Quecket Club) Freezing Microtome

replaced, must be firmly secured by the clamp screw for that purpose The substance to be cut is placed on the surface of the central circular brass piece, and surrounded with a solution of gum, which readily congeals, and will thus hold the specimen firmly in position,

which will solidify shortly after the gum has frozen It is advisable to cover the apparatus with baize, to facilitate the freezing process The edge of the razor must be elevated to the required height for cutting the section by means of the three screws supporting the frame After the first cut, each end of the razor must be again presented to the surface of the specimen, when either end of the blade can be adjusted by one of the back screws until its entire length is level , then by turning the large screw in the front part of the frame it can be lowered for each successive section required

Preparation of the Material for Freezing—Follow exactly the same methods as are indicated at p xxxviii A piece of tissue, a quarter of an inch in thickness, is quite sufficient to freeze at one time The tissue to be frozen is, after the above preparation, placed on one of the circular plates, and is fixed to it with gum solution The razor ought to be moistened with the same solution, which prevents the sections from curling up

TREATMENT OF THE SECTIONS

From the bowl of water, into which they are put at first, they are transferred to a conical glass filled with water, in which they gradually subside All the gum must be got rid of, which is accomplished by changing the water several times When all the gum is dissolved, transfer the sections to one or other of the following fluids till they are required *Keep them in glass-stoppered bottles*

PRESERVATIVE FLUIDS

1 Ordinary methylated spirit
2 Glycerine 1 oz , water 1 oz , carbolic acid 4 minims

DR BEVAN LEWIS'S ETHER FREEZING MICROTOME

It is not always possible to obtain ice for freezing, and hence it is necessary to have another medium for freezing This is supplied in the most perfect form by the above instrument (fig 21), devised by Dr Bevan Lewis, of the West Riding Asylum This microtome is specially valuable for *rapid* freezing

The lower half or body of the instrument (*a*) is a slightly modified Stirling microtome, the upper part (*b*) consists of a freezing-chamber and a section-plate

The Body — The framework of the body (*a*) is a solid brass casting, which can be secured to a table firmly by the coarse screw (*m*) Through its central aperture works the brass plug (*h*), driven by a spring and fine micrometer screw (*k*), the pitch being fifty threads to the inch, the screw having a diameter of $\frac{8}{10}$ths of an inch with a milled head $1\frac{1}{2}$ inches across

Section-Plate—The section-plate (*c*) is a circular plate of zinc $\frac{2}{5}$ths of an inch thick, raised upon a strongly vertical arm of brass or zinc—preferably of the latter metal In the section-plate there is a circular central aperture through which the freezing-chamber works Lately the efficiency of the instrument has been much improved by capping the zinc plate (*c*) with a square of plate-glass $\frac{1}{4}$-inch thick, extra polished and drilled with a central aperture (*n*) corresponding in size to that in the zinc plate, and which in my own instrument measures $2\frac{1}{2}$ inches in diameter For cutting sections of frozen tissue, the glass-section plate is a great advantage, and when once used will never be dispensed with It renders the movements of the knife absolutely free and easy, whilst, on the other hand, the blade never gets injured from scratches which sooner or later invariably appear upon a metal section-plate, and which turn or indent the edge

Freezing-Chamber —This consists of a zinc cylinder (*b*) 1½ inches deep by 2¼ wide, closed above and below An aperture (*f*), ¾ of an inch across, is made on either side of the cylinder to allow the admission of the nozzle of the spray-producer, as also for free evaporation of ether, a large proportion of which, however, condenses on the sloping false bottom (*g*) and is conveyed off by the tube (*l*) into a bottle attached to it

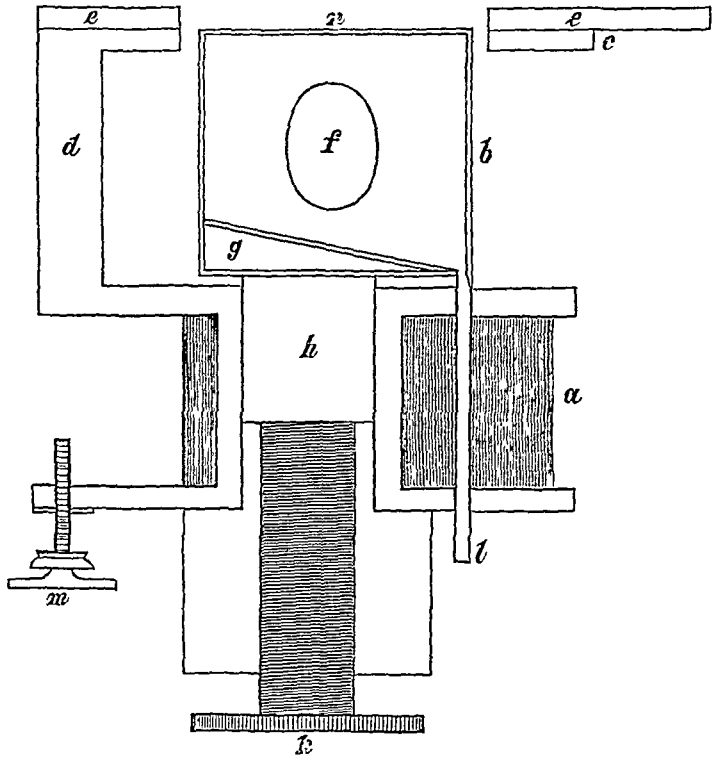


Fig 21 The Ether Freezing Microtome of Dr Bevan Lewis This instrument is made by Mr Gardner, South Bridge, Edinburgh

The instrument is simple, efficient, and compact, and enables one with a minimum expenditure of ether to obtain the most delicate sections of any tissue not more resistant than cartilage

A little experience in the use of the instrument enables the operator to freeze so rapidly that he will scarcely feel that it repays him to collect the condensed ether

METHODS OF STAINING TISSUES

TISSUES may be stained with certain reagents, *e g* eosin, which give a uniform colour to the section, whilst other substances derive their value from staining different parts of a section unequally, nuclei, for example, are usually stained of a deeper red than the surrounding protoplasm For the method of transferring large sections to a slide, see p xxvi

GENERAL DIRECTIONS

It is always advisable to use rather a weak solution of the staining agent, as this stains slowly and gives the dye time to completely permeate the section When practicable the sections should remain in the fluid for several hours The proper depth of tint can easily be ascertained by lifting out a section and washing it in water, and examining it on a slide with a low power A little practice soon enables one to tell when the staining is deep enough For class purposes, however, it is often necessary to use strong solutions of the dyes, and the results are very satisfactory

For class purposes staining with a strong dye is readily accomplished Float a section on to a slide (p xxvi), remove all the water from the slide and around the section Place on the section a large drop of the staining fluid, and leave it 'until the section is properly stained Wash off the surplus pigment in a bowl of water, float the section on to the slide again, and mount it in glycerine or other mounting fluid

STAINING SOLUTIONS

LOGWOOD SOLUTIONS

(*a*) A Dissolve 3 grms of hæmatoxylin in 10 c c of absolute alcohol B Dissolve 3 grms of alum in 100 c c water A few drops of A added to a few c c of B gives a solution of a beautiful violet colour, which rapidly stains tissues

(*b*) A strong infusion of logwood chips is made, to this reddish-coloured fluid, after filtration, alum is added, until a bluish-violet solution results The violet colour deepens by exposure to the air for several days

(*c*) Take 60 grms of extract of hæmatoxylin and 180 grms of alum Rub them together in a mortar and slowly add 280 c c distilled water Filter, and to the filtrate add 20 c c of absolute alcohol *Keep all of the above in glass-stoppered bottles Logwood solutions ought always to be filtered before they are used*

I have found solutions prepared by any of the above methods to give excellent results They act very rapidly on nuclei, giving them a bright violet colour, softer and pleasanter to the eye than the bright red stain of carmine Many, however, prefer a solution which contains

alcohol, such a solution is the following, which has the advantage of not requiring to be filtered so frequently

(*d*) *Kleinenberg's Logwood Solution*—Make a saturated alcoholic (70 per cent) solution of crystallised calcic chloride, and a similar solution of alum Add 8 parts of the alum solution to 1 of the calcic chloride To this mixture add, drop by drop, a saturated solution of hæmatoxylin in absolute alcohol, until the whole fluid assumes a deep purple colour Use only a small quantity of alcohol, as the hæmatoxylin is very soluble The colour deepens and improves by keeping When it is desired to stain a section, this solution must be diluted by adding a few drops of it to a watch-glass full of water

(*e*) *Logwood solution for rapid staining*—This solution, devised by E A Cook, yields good results, but it has no advantages over any of the above The ingredients are extract of logwood and alum, of each, 6 parts, cupric sulphate 1 part, and water 40 parts Grind in a mortar the alum, logwood, and cupric sulphate, all of which must be iron free, and when powdered add sufficient water to form a thin paste Leave this for one or two days, then add the remainder of the water and filter To the filtrate add a crystal of thymol, to preserve it from mould For tissues hardened in chromic acid add 8 drops of this solution to 120 drops of water, and add one drop of a tenth per cent solution of potassic bichromate just prior to use

CARMINE SOLUTIONS

Strong Carmine Solution for rapid staining—Rub up two grms of the best carmine in a mortar with a few drops of water, add 4 c c of liquor ammoniæ to dissolve the carmine, then add 60 c c of water Filter the solution and keep it in a stoppered bottle The solution may be diluted, if it be desired to stain a tissue slowly

Carmine is not employed so frequently as it used to be A strong solution, such as the above, is apt to give a somewhat uniform tinge to the section Better results are obtained by using either the above fluid diluted, or the following —

Weak Carmine Solution—Rub up 1 grm of best carmine in a mortar with a few c c of distilled water, add 3 c c of liquor ammoniæ, and, when the carmine is dissolved, add 150 c c of distilled water, and filter into a stoppered bottle Add 30 c c of glycerine and 100 c c of rectified spirit This is a modification of Beale's carmine fluid

All carmine-stained preparations, after being thoroughly washed in water, are improved by placing them for a few minutes in a one per cent solution of acetic acid This brightens the colour and fixes the carmine in the nuclei, and also differentiates the stained from the unstained parts The stain thus becomes more selective

PICROCARMINE, OR PICROCARMINATE OF AMMONIA.

Picrocarmine Solution—Make a solution of carmine by rubbing up 1 grm of best carmine with 10 c c of water, and adding 3 c c of liquor ammoniæ Add this solution to 200 c c of a cold saturated solution of picric acid Leave the mixture exposed to the air until it evaporates to one-third of its bulk The same result may be obtained by evaporating it slowly in a hot chamber, or over a water-bath at a low temperature Filter to get rid of the precipitated carmine

This substance was introduced by Ranvier, and is one of the most valuable dyes one can employ It is especially used for 'double staining,' containing as it does two pigments, one yellow and the other red—*e g* the perinuclear part of a frog's coloured blood-corpuscle is

stained yellow, the nucleus red Connective tissue is stained red and elastic tissue yellow It has this further advantage—that though sections are left for several hours in the solution, they seldom become overstained It is not necessary to remove all the picrocarmine from the section, in fact, it is advisable to leave a little adhering to the section, for after being mounted in glycerine or Farrant's solution, the trace of dye left is gradually absorbed by the section If it be desired to retain the yellow and red colours it is desirable *not to wash* the section after staining, because the yellow picric acid stain is readily removed thereby In some cases it is advisable to mount the section in glycerine, containing one per cent of formic acid

All picrocarmine preparations improve by keeping After several days nuclei become stained that at first were unaffected, so that sections should invariably be examined several days after they are mounted

Picrocarmine may be purchased as an imperfectly crystalline body If the crystals are used, make a one per cent solution

Purpurine—The dye obtained from madder has no advantages over any of the other methods It can be dispensed with

ANILINE DYES

(*a*) **Magenta Solution**—Dissolve 1 decigrm of the sulphate or nitrate of rosanilin in 100 c c of water, and add 10 c c of rectified spirit This does very well for fresh tissues The stain is not permanent, though it may last for many months For *blood-corpuscles* 2 decigrms are dissolved in 40 c c of a mixture of equal parts of water and glycerine

(*b*) **Aniline Blue, soluble in Water**—Make a one per cent solution in water This is sometimes used for fresh tissues

(*c*) **Aniline Blue-Black**—Make a one per cent solution in water If the section is overstained the excess may be removed by steeping it in a two per cent solution of chloral hydrate *For the nervous system* an alcoholic solution is most useful Dissolve 1 decigrm in 4 c c of water, add to this 100 c c of rectified spirit and filter Preserve in a stoppered bottle This solution stains rapidly and gives a pleasant slate-grey colour, which fatigues the eye very slightly It is specially useful for nerve-centres Sections may be mounted in dammar without any fear of removing the dye

(*d*) **Methyl-Aniline**—Make a strong watery solution and stain the tissue deeply Wash away the superfluous pigment in water acidulated with acetic acid The acetic acid dissolves out much of the pigment, and the washing must be continued till the proper tint is obtained The sections must then be thoroughly washed in water, and mounted in glycerine or Farrant's solution Dammar is unsuitable, as clove oil discharges the colour

This is a most valuable dye, for in contact with certain tissues it gives a 'double-stain' It decomposes into two colours—one a *red-violet*, the other a *blue-violet*—each of which acts on different tissues It is useful for hyaline cartilage, the red-violet attaches itself to the matrix, and the blue-violet to the corpuscles

It is in the pathological change known as *amyloid or waxy degeneration*, however, where it is most useful All parts of an organ—*e g* the liver—affected with amyloid degeneration are stained red-violet, whilst the neighbouring unaffected elements are coloured blue-violet

For *fresh tissues* it also forms a useful dye, and in the form of a one per cent watery solution it may be used instead of magenta It stains certain parts of a beautiful violet It is very useful for showing the corpuscles in connective tissue, or the nuclei in fresh cells, or for mucous tissue It is best to mount a tissue in a saturated watery solution of potassic acetate

(*e*) **Iodine Green** —A saturated watery solution may be made and diluted as it is required, or a one per cent solution may be prepared It stains rapidly, and the stain cannot be removed by washing It is one of the most useful of the aniline dyes, especially for double-staining (p xlvi) It stains mucous glands green, and acts similarly on unossified cartilage Preparations stained with it must be mounted in dammar Do not leave the stained sections long in spirit, which partially removes the dye

(*f*) **Rosein** is soluble in spirit, and so is useful for double-staining when the sections are to be mounted in dammar See retina Ordinary methylated spirit does quite well as a solvent

Many other aniline dyes are employed, but the above are certainly the most useful

(*g*) **Eosin**, though not an aniline dye, may for convenience be taken here It yields a rose-coloured dichroic fluid, when dissolved Dissolve 1 part in 1,500 of water Tissues become stained in a few seconds After staining, place them in water slightly acidulated with acetic acid (one per cent), to remove the excess of pigment and to 'fix' the remainder Sections may be examined either in glycerine or in dammar For some purposes, as for staining the hæmoglobin of coloured blood-corpuscles, it is used as an alcoholic solution It is very useful in the study of developing coloured blood-corpuscles

METALLIC SOLUTIONS.

(*a*) **Silver Nitrate** —Dissolve 1 grm of silver nitrate in 100 c c of distilled water For use dilute this to $\frac{1}{3}$ or $\frac{1}{2}$ per cent solution Wash the tissue to be stained (*which must be quite fresh*) in distilled water to remove chlorides, place it in the silver solution for five minutes, or until it becomes of a whitish appearance, then remove it, wash it in ordinary water, and expose it in water, or alcohol and water, to diffuse daylight till it becomes brown in colour

Silver nitrate solution is used where it is desired to bring into view the existence of epithelial cells—*e g* on serous membrane, lining arteries, lymphatics, capillaries, or the air-cells of the lung It acts upon the *intercellular substance*, or *cement*, and when the tissue is exposed to the action of light, it is precipitated in the intercellular substance as the black oxide, in fine black lines, and thus the cells are mapped one from another These lines are known as 'silver lines' It also stains the intercellular substance of cartilage and the cornea The preparations may be preserved in glycerine or dammar, and ought to be kept in a dark place, as they are apt to spoil when long exposed to light

(*b*) **Gold Chloride** —Solutions from $\frac{1}{2}$ per cent to 2 per cent are employed

1 Place the perfectly fresh tissue in the gold solution ($\frac{1}{2}$ per cent) for twenty to thirty minutes, wash it in water, and then expose to daylight in water slightly acidulated with acetic acid (2 or 3 drops of acetic acid to 1 oz of water) until it becomes of a purplish colour

2 Or the following method may be employed, especially where the tissue is dense and it is not desirable to retain the epithelium Cut a fresh lemon in two, squeeze out the juice and filter it through muslin Place the perfectly fresh tissue—*e g* cornea—in the juice for five or seven minutes, wash it in water to remove the juice, and steep it in a one per cent solution of gold for twenty minutes, or half an hour Wash off the surplus gold, and place the object in a mixture of one part of formic acid to four of water Put the bottle in a dark place After twenty-four hours the gold is completely reduced All the epithelium, however, is removed by the formic acid This method is the best for demonstrating the terminations of nerves in muscle

Gold is especially useful for staining nerve-fibrils—*e g* in the cornea—and also for connective tissue-corpuscles

Under each tissue where gold is useful, special directions are given for its employment

(*c*) **Osmic Acid**—Make a one per cent solution which can be diluted as required—to $\frac{1}{4}$ or $\frac{1}{2}$ per cent The method of using and preserving this substance has already been referred to under Hardening Solutions (p xxxii) It is useful for blackening fat-cells, and for fixing the white substance of Schwann in nerve-fibres, though it has many other important applications

ON DOUBLE-STAINING OF TISSUES

By using two differently coloured solutions, it is found that in certain tissues one part will take up one colour, whilst another part takes up another Some glands stain of a green colour with iodine-green—*e g* the mucous glands of the tongue—whilst the other glands, the serous glands, are not affected by it

This method is of the greatest value, and I find it can be practised very successfully by students even in large classes

PICROCARMINE

As already indicated, this dye stains certain parts yellow and other parts red, hence its value

PICROCARMINE AND LOGWOOD

Stain the sections slightly with picrocarmine, and after washing them in water, place them in dilute logwood solution, in which they must not remain too long They soon become lilac-coloured Wash them and mount them This method is useful for the mesentery of the newt (p 38), for developing bone, epiglottis, &c

PICROCARMIME AND IODINE GREEN

This is one of the most useful combinations I know Stain the sections in picrocarmine, and wash them well in water slightly acidulated with acetic acid, and then stain them in a watery solution of iodine-green (p xlv), taking care that they do not become over-stained, which can easily be ascertained by washing them in water If a section of the posterior third of the tongue be so stained (p 61), all the muscles and connective tissue are red, whilst the mucous glands and adenoid tissue are green The serous glands do not take up the green stain This combination is, therefore, of the utmost value for gland-tissue All the mucous glands and adenoid tissue of the intestinal tract become green, whilst the connective tissue is red, and the non-striped muscle of a light yellow or brown Most exquisite effects are produced in the cerebellum, bone, and intestine by this method The sections must be mounted in dammar

EOSIN AND LOGWOOD

Stain the sections lightly in eosin and then in logwood This does very well for the brain

GOLD CHLORIDE AND SILVER NITRATE

This method is sometimes employed for the cornea, which is first impregnated in the usual way with gold chloride and then with silver nitrate The method is very difficult to be successful with and often fails

TREBLE STAINING

This method has recently been practised by Dr Heneage Gibbes The process is tedious, and not so satisfactory as double-staining A combination of picrocarmine and iodine-green for sections of the small intestine, including a Peyer's patch, gives an excellent and most instructive effect (p 69), in fact a perfect treble stain

Other combinations, as picrocarmine, eosin, and iodine-green, have been tried

GOLD CHLORIDE AND AN ANILINE DYE

This process is alluded to in connection with the study of the parts in the tail of a rat (p 25) The tissue is first treated with gold chloride and then decalcified, and the sections are stained with rosein, and then with iodine-green, or with iodine-green alone The effects produced on the developing bone especially are most remarkable and striking This method is capable of further extension

LIST OF STAINING REAGENTS

Ordinary staining reagents

1 Logwood or Hæmatoxylin
2 Carmine
3 Picrocarmine
4 Eosin

Aniline dyes soluble in water

1 Magenta, or Rosanilin
2 Aniline blue
3 Aniline blue-black
4 Methyl-aniline
5 Iodine Green

Aniline dye soluble in spirit

Rosein

Metallic Substances

1 Silver Nitrate
2 Gold chloride
3 Osmic Acid

Many other dyes, as iodine, indigo-carmine, &c, are recommended, but the above are what the author has found to be really useful

METHODS OF MOUNTING SECTIONS

MOST sections, even when very thin, are too opaque to be examined, and must therefore be placed in some medium or mounting fluid which will render them more transparent

MOUNTING FLUIDS

1 *Glycerine*—The section must be floated on to a slide from water, and, after removing the water, a small drop of glycerine is applied The drop ought to be of just such a size that when the cover-glass is applied, it accurately fills the space between the cover and the slide Apply a cover-glass, as directed (p xxiv) A little practice is required to gauge the size of the drop required If too much glycerine be added, the excess is most conveniently removed by placing several small pointed pieces of bibulous paper round the margin of the cover *All surplus glycerine must be carefully removed,* else the cement to be afterwards applied will not adhere to the glass Glycerine has this great disadvantage, that the cover-glass moves so easily that it becomes difficult to seal up the preparation This is entirely obviated by mounting sections in the following fluid, which has the advantage, that after exposure to the air for three or four days, the cover-glass becomes firmly fixed to the slide, so that the coating of cement can be applied without any fear of displacing either the cover or the section

2 *Farrant's Solution*—Take equal parts of glycerine and a saturated watery solution of arsenious acid Add gum arabic in large quantity, and allow the mixture to stand for several weeks, stirring it from time to time, until it ceases to dissolve more of the gum Filter through paper This takes a considerable time, but one is rewarded by the beautifully clear fluid obtained

The above fluids are suitable for mounting sections—stained or unstained—from water Unless for very delicate tissues, such as isolated epithelial cells, Farrant's solution is in all cases to be preferred It is always advisable, when wishing to study the minute relations of parts—except those of the nerve-centres—to mount sections in either of the above fluids

3 *Glycerine Jelly*—This is sometimes employed to mount softened bone and tooth, and some urinary deposits It must be melted in a water bath before being used As it sets rapidly, the cover-glass must be applied at once to the preparation It is far better to buy it ready-made than to take the trouble of making it Rimington's is the best it contains, in addition to gelatine and glycerine, a trace of carbolic acid

4. *Damman Solution*—Mix in an earthenware vessel 2 oz of mastic and 2 oz of dammar with 2 oz of chloroform and 2 oz of turpentine Shake or stir the mixture from time to time until the substances are dissolved Filter through paper Formerly Canada balsam was employed, but the above fluid is certainly to be preferred If Canada balsam be thought preferable, it may be easily prepared by heating some balsam in a capsule in a warm chamber at 65° C for twenty-four hours, and, after it cools, dissolving the dry yellow mass in as much benzole as will give a fluid of a syrupy consistence

Both dammar and Canada balsam render tissues much more transparent than glycerine

All traces of water must be removed from the sections before they can be mounted in dammar or Canada balsam This is done by immersing them for five or ten minutes in absolute alcohol

METHOD OF MOUNTING IN DAMMAR

When a section is to be mounted in dammar *all water must be got rid of* Place the section in absolute alcohol for five or ten minutes, until all the water is removed Transfer it for the same period to oil of cloves or turpentine,—preferably the former, which expels the alcohol, and renders the section quite transparent *These operations can all be accomplished on a slide thus*,—Float out the section on to the slide in water (p xxvi), remove the water with blotting-paper, add absolute alcohol to remove the remainder of the water, after a few minutes soak up the alcohol with blotting-paper, and with a stiff brush insinuate a drop of oil of cloves *under* the section The clove oil gradually rises through the section, expels the alcohol, and renders the section quite transparent This process ought to be observed with a low power under the microscope Remove the surplus clove oil with blotting-paper, add a drop of the dammar mounting fluid, apply a cover, and the preparation is finished.

LABELLING AND PRESERVING SECTIONS

After mounting, each section must be properly labelled (p xxvi), and laid *flat* in a tray, and preserved from dust

METHODS OF SEALING UP THE PREPARATIONS FOR PRESERVATION

Each preparation has still to be sealed This is done by placing some kind of cement over and round the margin of the cover-glass After several days the specimens mounted in dammar and Farrant's solution will be ready for sealing up This is best done by means of a *turntable*, which consists of a brass disc, at least three inches in diameter, and rotating on a pivot The upper surface of the disc has on it a series of concentric rings, corresponding to the different sizes of cover-glasses used The disc is rotated by means of the forefinger of the left hand acting on a small milled head placed underneath The slide is placed on the disc, accurately centred and fixed in position by two clips, which ought to be made of bent steel spring in preference to brass A camel-hair or goat-hair brush is dipped in the cement, and whilst the disc is rotated a ring of the cement is run *round* and *over* the margin of the cover This ought to be done twice or thrice, until a sufficiently strong ring is formed In selecting a turntable, see that the disc is heavy and wide enough to admit the largest slide under the clips The disc must be heavy, and should rotate steadily and easily, and this is best secured by having the pin or pivot on which it rotates made

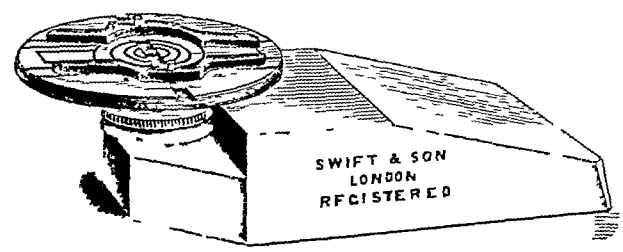


Fig 22 MODIFIED TURNTABLE

'*pin-pointed*,' *i e* the upper half of the pin much finer than the lower half When square cover-glasses are used, the cement is simply painted on with a brush

Fig 22 shows a turntable invented by Mr Dunning It is so made that any non-central position may be obtained, even with slides two inches in width, thus facilitating the application of the cement to any slide the cover-glass of which has not been accurately centred

I METHODS FOR PREPARATIONS MOUNTED IN DAMMAR

It is not necessary to apply any cement, but the preparation looks neater if a ring of the ordinary dammar mounting fluid is run round the margin of the cover-glass After using the brush wash it in benzole to remove the dammar

II FOR PREPARATIONS MOUNTED IN GLYCERINE OR FARRANT'S SOLUTION

After removing all the surplus mounting fluid, paint a ring of *marine glue* or *painter's gold-size* round the cover-glass, so as to fix it down, and after it dries (twenty-four hours) put on a *thick* ring of *zinc-white cement* It may be necessary to put on more than one ring of zinc cement This is by far the best way of sealing up these preparations When the zinc-white dries it forms a ring as hard as enamel The brush used for the zinc-white ought to be washed at once in benzole, and the one used for gold-size in turpentine, and the one for glue in water These simple precautions ought never to be neglected

Various other substances, as asphalt and melted paraffine are recommended for sealing up preparations, but none of them are equal to the above described method

The æsthetically inclined student can easily tint the white cement by adding to it a little solid eosin or any other dye It is, however, quite unnecessary to spend time in adorning the outer surface of preparations

FLUIDS FOR SEALING UP PREPARATIONS

1 The ordinary *damma*r solution may be used

2 **Zinc-white Cement**—(Gum dammar, 8 oz , zinc oxide, 1 oz , benzole, 8 oz Dissolve the dammar in the benzole and add the zinc oxide, and then strain through muslin)

3 **Marine Glue** (Hollis')

4 **Painter's Gold Size**

Number 3 or 4 is applied first, and when it becomes dry it is covered with a ring of zinc-white cement In my opinion gold size is preferable to marine glue It is not so liable to crack

METHODS OF INJECTING THE BLOOD-VESSELS

The ordinary method of hardening tissues in chromic acid does not suffice to reveal the arrangement of the blood-vessels of a part, hence we must have recourse to filling the blood- or lymph-vessels artificially with a coloured mass The coloured fluids at present employed are of two kinds—one kind which is *fluid*, the other which is *solid at ordinary temperatures* The chief medium of the former is water or water and glycerine, and of the latter gelatine

INJECTION FLUIDS

(A) INJECTION-MASSES FLUID AT ORDINARY TEMPERATURES

1 **Watery Solution of Brucke's soluble Berlin-blue**—Dissolve 10 grms of Brucke's soluble blue in 500 c c of distilled water

Brucke's soluble blue can be purchased in the market, but if it be desired to make it, the following is the formula The process is rather tedious (*a*) Dissolve 217 grms of potassic ferrocyanide in 1 litre of distilled water (*b*) Make one litre of a 10 per cent solution of ferric chloride Add to each of these solutions (*a* and *b*) two litres of a saturated solution of sodic sulphate Then add the ferric chloride to the ferrocyanide, and stir the fluids whilst they mix The precipitate is filtered through a conical bag of new flannel, and washed with a small quantity of water The first washings are returned The washing is repeated for several days, until the solution when passed through filter paper is of a deep blue colour It is then soluble The precipitate is collected from the flannel, pressed between folds of blotting paper, dried, and broken into small pieces After the blood-vessels of an organ have been filled with this solution it is placed in equal parts of methylated spirit and water, to which a trace of hydrochloric acid is added, as this prevents the diffusion of the blue, or the organ may be hardened in Muller's fluid or picric acid In some cases it is advisable to heat the injection-fluid to a temperature of 40° C before injecting it into the vessels A little glycerine added to the mass makes it flow more easily

2 **Richardson's Blue**—(*a*) Dissolve 10 grains of ferric sulphate in 10 oz of water (*b*) Dissolve 32 grains of potassic ferricyanide in 1 oz of water Mix *a* and *b*, and then add water to 20 oz

(B) INJECTION-MASSES NOT FLUID AT ORDINARY TEMPERATURES

1 **Soluble Blue and Gelatine**—Soak 50 grms of the best gelatine (Cox or Coignet) in water for several hours Pour off the water which is not absorbed by the strongly swollen-up gelatine, and melt the gelatine over a water-bath The gelatine may be placed in an ordinary jelly can or tin vessel, and this placed in a pan of water on the fire, if a water-bath is not at hand A two per cent watery solution of Berlin-blue is prepared (p li), and heated to the same temperature as the gelatine Take 250 c c of this fluid, and gradually add to it the solution of gelatine, the mixture being stirred all the time. Whilst still hot the mixture is filtered through flannel, and then kept at a temperature of 40° C until it is injected After the injection is finished, place the organ in alcohol for twenty-four hours, to coagulate the gelatine

2 **Carmine and Gelatine Mass**—(*a*) Take 1 oz of the best gelatine, and make a solution of it as described above Strain while hot through flannel, and make up the solution to 2 oz (*b*) Place 1 drachm of the best carmine in a mortar, to it add 1 drachm of liquor ammoniæ and 2 oz of water, and leave it for twelve hours (if in a hurry, place the carmine solution near the fire for an hour) Filter, and add about 80 minims of glacial acetic acid, drop by drop, stirring all the while, until the ammonia is completely neutralised As the odour of the ammonia becomes faint the acid must be added very cautiously As long as there is free ammonia the fluid is of a dull red, but it becomes of a bright, florid, cherry colour the moment the ammonia is neutralised Add the florid red carmine fluid (*b*) to the 2 oz of gelatine solution (*a*), and keep at a temperature of 40° C until the mass is required

The principle to be attended to in making the mass is this —The carmine, if alkaline, would diffuse through the tissues, if acid, it would be deposited in fine granules, which block up the capillaries—hence the necessity for having a neutral fluid The best guides are the colour and odour of the fluid The injected organ must be placed in equal parts of methylated spirit and water, to which a few drops of acetic or hydrochloric acid (one per cent) are added, to prevent the carmine from diffusing into the tissues

APPARATUS EMPLOYED FOR INJECTING THE BLOOD-VESSELS

Injections are made either with a syringe, or with some form of apparatus, which will give a constant pressure Whatever apparatus be employed it is necessary to insert a cannula into the blood-vessel by which the injection will flow, *e g* the aorta The cannulæ may be made of brass, such as are ordinarily supplied with a syringe, or they may be made of glass, with a short piece of india-rubber attached to them The advantage of a glass cannula is that one can see when air-bubbles are present For the method of making these cannulæ see fig 23

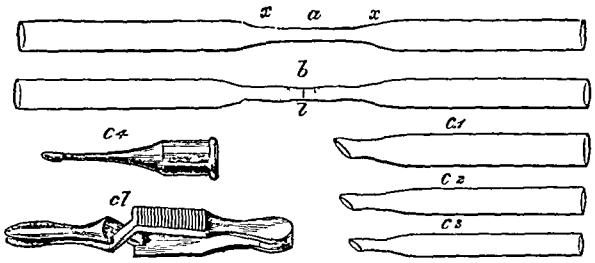


Fig 23 Cannulæ for Injecting Natural Size

c^1 c^2 c^3, Glass Cannulæ of different sizes, *a* and *b* show the method of making a cannula a glass tube is heated in a flame and drawn out to the size required, as at *a* It is then heated in the middle and slightly drawn, to make it thinner there After it cools it is cut by means of a triangular file at *l*, and then each half is ground down obliquely on a hone The sharp edges are got rid of by heating it in a flame for a few seconds A piece of india rubber tubing is tied to the end of each and the cannula is complete c^4, A metal cannula, *cl*, A steel clamp for clamping a small artery (Schafer)

The cannula ought to have a shoulder, over which the thread is applied, so that when tied it cannot slip out of the vessel

Method of inserting a Cannula into a Blood-vessel — Expose the artery of the part to be injected, clear a small piece of it from the surrounding tissue, and place a ligature around it by means of an aneurism-needle, or a narrow-pointed pair of forceps With sharp scissors make an oblique slit in its wall, and insert the nozzle of the cannula, directing it towards the periphery Tie the ligature firmly over the artery, and behind the shoulder on the cannula

HOW TO INJECT BY MEANS OF A SYRINGE

The syringe is made of brass, and is provided with several cannulæ, of different sizes, and a separate piece with a stopcock to connect the cannula with the syringe Tie the cannula in the vessel, as directed above, and fill it with salt solution, by means of a pipette Fix the stopcock-piece into the cannula, and fill it in the same way with salt solution Then fill the syringe with the injection-mass, taking care not to suck up any air All air ought to be expelled from the syringe by holding it vertically, nozzle uppermost, and expelling a few drops of the injection-mass The syringe ought to be filled and emptied several times before doing this, to get rid of air Insert the syringe in the stopcock-piece, and proceed slowly with the injection The piston must be *rotated very slowly*, so that the mass is driven on slowly and steadily Half-an-hour is not too long for the injection of a rabbit One judges of the completeness of the injection by observing the colouration of such vascular parts as the tongue, lips, toes, or ear After the injection is completed, turn the stopcock to prevent the escape of fluid, remove the syringe, and place the part injected in equal parts of alcohol and water, or any of the other fluids described at p xxxii If a gelatine mass be employed, it is better to

inject the animal after it has been killed by bleeding The animal or part to be injected must be kept warm by immersing it in water at 40° C Even when a watery injection is employed, it is as well to warm it to 40° C, as while hot it causes less spasm of the small arteries than a cold mass

The art of injecting can only be acquired by practice The part to be injected and the mass must be at the proper temperature, no air must be allowed to enter the blood-vessels, and the mass itself must not contain any particles which will block up the capillaries One of the great difficulties is to keep the pressure constant, this may in part be arrived at by fixing a tube at right angles to the side of the stopcock-piece, and bringing in connection with it, by means of a caoutchouc tube, an ordinary mercurial manometer, viz, a bent tube filled with mercury A pressure of two to five inches of mercury is usually sufficient for injecting small animals

In selecting a syringe see that you get one that is capable of holding from three to six ounces The barrel ought to be *long*—six or seven inches For corrosive (or metallic) substances, a glass or vulcanite syringe must be employed

INJECTING APPARATUS FOR OBTAINING A CONSTANT PRESSURE

Ludwig was the first to introduce this arrangement Sufficient pressure may be obtained either by means of a column of mercury or a column of water

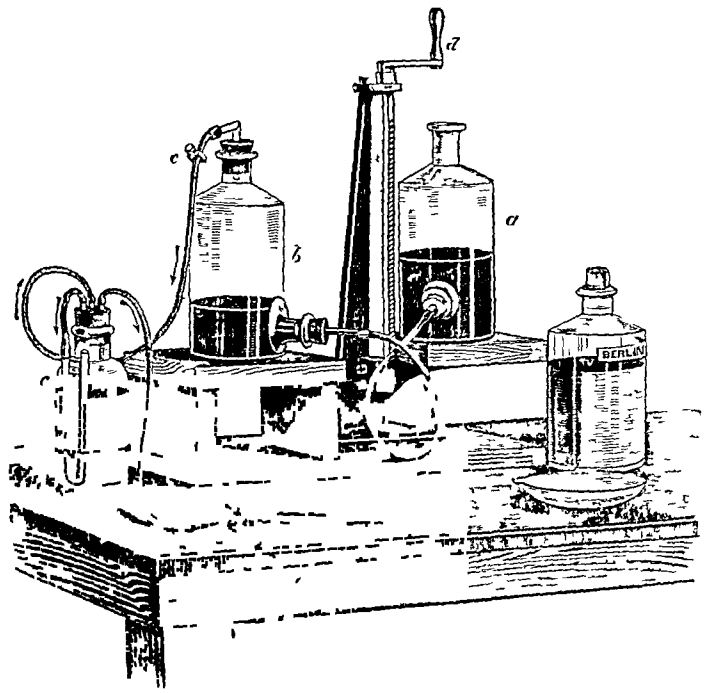


Fig 24 Ludwig's Mercury Pressure apparatus

a, Reservoir bottle for mercury, *b*, Pressure bottle into which the mercury of *a* flows, *c*, Bottle containing the injection mass, connected with *b* by one india rubber tube, and by another with the cannula *f*, and by a glass tube with a small manometer, *d*, Handle of screw for raising the wooden shelf on which *a* rests, *e*, A screw clip, and *g*, a spring clip

Ludwig's Mercury Pressure-apparatus (fig 24)—The apparatus consists of two Wolff's bottles, one of which, *a*, contains a reserve of mercury, and *b*, into which, owing to the bottles being at different levels, the mercury flows This compresses the air in *b*, and so acts on the

injection-fluid contained in the bottle *c*, to which a mercurial manometer is attached From this an exit caoutchouc tube passes, which is attached to the cannula fixed in the artery The pressure-bottle *a* rests on a wooden support, which is raised by means of a screw, and in this way sufficient pressure is obtained In injecting with any pressure-apparatus, begin with a pressure of one inch of mercury, and slowly increase it to four inches Of course if a gelatine mass be employed the bottle *c* must be kept warm, by placing it in warm water to keep the gelatine in a fluid condition

Before beginning the injection, the cannula and the exit-tube from *c* must, of course, be filled with the injection-mass, to avoid the entrance of air This apparatus is expensive, and therefore an apparatus that one can make for himself is to be preferred, such as the following simple mercurial injection-apparatus (fig 25) It works on exactly the same principle, and

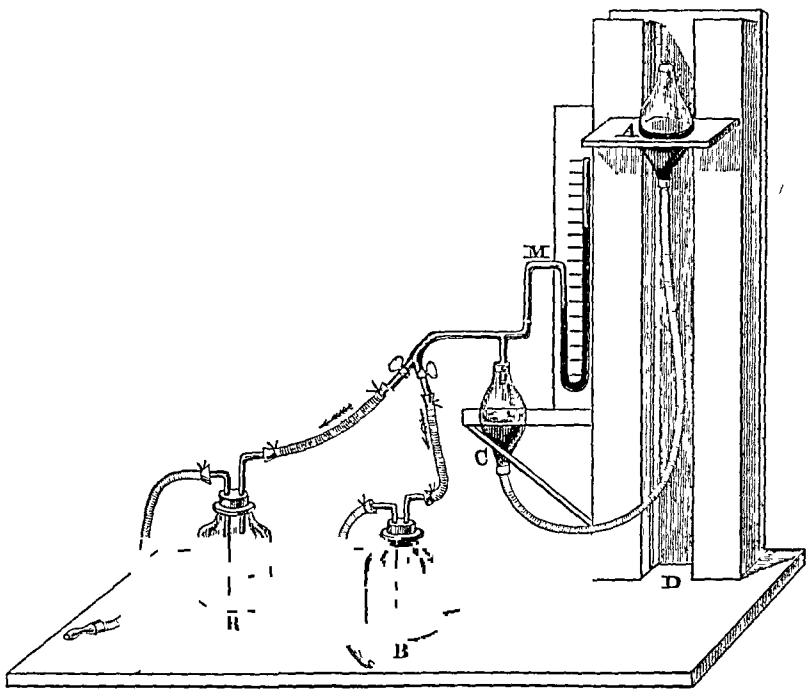


Fig 25 Simple Mercurial Injection apparatus

fig 25 explains itself One of the bulbs (C), containing the mercury, is stationary, whilst the other (A) rests in a wooden framework, or shelf, which moves freely in a slot (D)

The part of this movable shelf which is fixed in the slot is so made that it works very freely, and when it is loaded at the free end of the horizontal arm, it becomes self-fixing at any height The pressure is very easily regulated by raising or depressing the movable shelf (A) By means of a divided, or Y-shaped tube, two injection-fluids (B, R) may be used simultaneously This arrangement, I find, works admirably

Simple Water Pressure-apparatus—Mercury is not always to be obtained, hence it is sometimes convenient to employ water-pressure Satisfactory results are not obtained by connecting a water-tap to a system of pressure-bottles The following arrangement (fig 26) is simple, cheap, easily made, and effective It consists of a bottle, C, which holds the injection mass, a much larger glass bottle, B, with a manometer (M) attached, and communicating

with this is a cylinder of tin (A), which is filled with water, and is raised or lowered by means of a cord which passes over a pulley (P) fixed to the ceiling of the room

INJECTION OF THE BLOOD-VESSELS OF AN ENTIRE ANIMAL

Having determined which injection-mass is to be used, prepare the cannulæ The animal —a rabbit or guinea-pig—whose blood-vessels are to be injected, is killed by chloroform As soon as it ceases to breathe, the thorax is opened by making an incision along the middle of the sternum, so as to expose the heart Pull the heart forward, and open the pericardium Snip through the wall of the right auricle, and allow as much blood to flow out as possible, the animal may even be suspended by the hind limbs to expel as much blood as possible Expose the aorta, and tie into it a glass cannula (fig 23, *c*) as directed at p lii The cannula is now filled with salt solution, and is connected either with a syringe or one of the constant-pressure arrangements described above Take care that there is no air either in the cannula or in the india-rubber tube coming from the injection-bottle At first a pressure of two inches of mercury is sufficient, but gradually the pressure may be raised to four inches As the injection is forced into the aorta, and gradually fills the blood-vessels, blood and then blood and injection-mass flow out of the right auricle Continue the injection until the injection alone flows from the hole in the right auricle It is then closed by a broad ligature, and the injection continued until the vascular system is filled with the injection, which may be judged of by the colour of transparent parts, as the ears, lips, &c After the injection is complete, ligature the vessels at the base of the heart, or a glass stopper may be put into the caoutchouc tube attached to the glass cannula The after-treatment of the injected animal is described at p lii

If an animal be allowed to cool after death, it must be thoroughly warmed before using a gelatine mass This is done by placing it for an hour in warm water not above 40° C

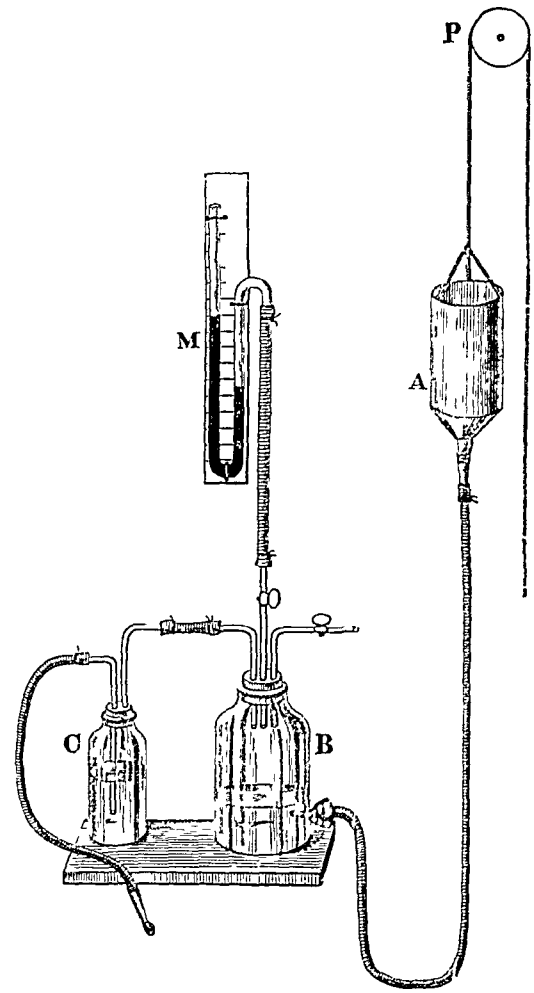


Fig 26 Injection Apparatus with Water Pressure

A, Vessel filled with water, B, Bottle for compressed air, C, Bottle for injection mass, M, Manometer

INJECTION OF THE LYMPHATICS

Puncture Method—This is very easily done by the 'einstich' or 'puncture' method of Ludwig A small subcutaneous (Pravaz) syringe (fig 27, p 22) is filled with a two per cent

solution of Brucke's blue (p li), and the nozzle is thrust into the *pad of a dog's or cat's foot*, and the fluid injected into the pad, which is then compressed, and the limb stroked from below upwards The coloured mass finds its way from the connective-tissue spaces into the lymphatic vessels, and runs along them very easily In this way one can easily inject the glands of the leg and abdomen, and even the thoracic duct The lymphatics of the *testis* are injected in the same way It is easy to demonstrate the lymphatics in the *intestinal* wall and in the liver by the same method Instead of a syringe, one of the constant-pressure arrangements may be used

PRACTICAL HISTOLOGY.

BLOOD

WHEN examined microscopically blood is seen to contain corpuscles floating in a fluid—the plasma or liquor sanguinis The corpuscles are of two kinds, (1) coloured and (2) colourless, and in addition (3) minute granules are to be seen Besides these, there fall to be examined (4) the fibrin and (5) the colouring matter of the coloured corpuscles and (6) various pigments derived therefrom

(A) BLOOD OF A FROG OR NEWT

PREPARATION—Give a frog or newt a smart blow on the head If a newt be taken, carefully wipe its tail dry by means of a cloth and snip off the point of the tail Then squeeze out a drop of blood from the stump on to a glass slide, and at once apply a cover-glass If a frog be used a toe should be cut off, when a drop of blood can easily be squeezed out of the stump In either case the blood thus obtained will be mixed with a considerable quantity of lymph If a drop of blood without any admixture of lymph is required, the heart of the animal must be exposed, and when this is done the auricle should be snipped through and a drop of blood allowed to fall on the glass slide

EXAMINATION—1 Observe the **coloured corpuscles** (H) They are seen to be very numerous, of a yellowish straw colour, uniform in size and elliptical in shape when seen on the flat (Pl I, Fig 1)* A few of them may also be seen edgewise, and they then appear narrow, somewhat pointed at the ends, and slightly thicker in the middle (Pl I, Fig 2) Sometimes one corpuscle lies above another, when the outline of the undermost one may be distinctly seen through the one lying above it, it is thus seen that each corpuscle is transparent At first each corpuscle may seem homogeneous, but in a very short time an elliptical slightly granular body shows itself in the centre of each as seen on the flat This body, which is somewhat lighter in colour than the rest of the corpuscle, is called the *nucleus* (Pl I, Fig 1)

EFFECTS OF REAGENTS on the coloured corpuscles

(*a*) **One per cent Hydrochloric Acid.**—Squeeze out a fresh drop of blood on a clean slide, quickly apply a cover-glass, and then place a drop of a one per cent. dilution of hydrochloric acid at one edge of the cover-glass (taking care that none of the acid gets on the top of the cover-

* Where a simple reference is made to a plate and figure (as Pl I, Fig 1), it is intended that the student shall insert by delineation and colouring such appearances and changes as are indicated in the text as the result of reagents or otherwise.

glass) Place a small piece of blotting-paper on the slide at the edge of the cover-glass opposite to the spot where the drop of acid was placed, which will have the effect of drawing the acid under the cover so as to 'irrigate' the blood-corpuscles with the reagent Steadily observe one or two corpuscles , they will gradually enlarge, become globular and all of a sudden swell up and become distinctly spherical, and then as suddenly collapse and 'shoot' or discharge their hæmoglobin The nucleus comes clearly into view during the process This process ought to be diligently looked for, because the phenomenon is so striking It is as if the corpuscle burst and discharged something After the collapse, the residue of the stroma adheres to the nucleus In other cases the corpuscles do not collapse, but remain globular, clear, and transparent The peri-nuclear part becomes decolourised, and appears to be limited externally by a membrane from which fine shreds—the residue of the stroma—stretch to the nucleus, which is now clearly brought into view, and has assumed a distinctly granular appearance, due to the existence of a fibrillar network in its interior If the acid used be too strong this action may be missed, and the outline of the corpuscles becomes very faint, and the shrivelled nucleus may appear excentrically, or may even escape altogether outside the corpuscle These phenomena are best observed with frog's blood

(*b*) **Acetic Acid.**—Shows the same phenomena, though in a less degree The corpuscles swell up and become clear, so that their nucleus becomes very obvious, and the nucleus not unfrequently passes out of the corpuscles It is to be noted that all the corpuscles are not so affected, which is perhaps due to difference in the chemical constitution of the corpuscles In many corpuscles distinct vacuoles are formed in the peri-nuclear part The coloured pigment may be seen gradually to leave the wall of the corpuscle, and become heaped up round the nucleus The fluid in which the corpuscles float becomes slightly coloured by a diffusion of the pigment of the blood-corpuscle (hæmoglobin), and sometimes the nucleus takes up some of this pigment and becomes of a distinctly yellow colour (Pl I , Fig 3)

(*c*) **Water**—Squeeze out another drop of blood on to a slide, cover, and irrigate with water in the manner just described The corpuscles and their nuclei both swell up and become globular, owing to endosmosis, and at the same time the hæmoglobin diffuses into the surrounding fluid (Pl I , Fig 6)

(*d*) **Syrup**—Squeeze out a fresh drop of blood, add a drop of **strong** syrup of white cane sugar, and mix the two drops thoroughly with the point of a needle, and apply a cover-glass Owing to the greater specific gravity of the syrup the corpuscles lose fluid, and, on examination some of them present a shrivelled appearance, and resemble thin misshapen biscuits, while others are but slightly affected Here and there a reddish tinge is seen in a corpuscle (Pl I , Fig 4)

(*e*) **Dilute Alcohol**—One part of rectified spirit to two of water, applied in the same way as acetic acid, decolourises the corpuscles, causes them to swell, and brings a nucleolus into view (usually placed towards one end) in each nucleus (Ranvier, Stirling)

(*f*) **Magenta**—Mix a drop of blood with a drop of a solution of sulphate or nitrate of rosaniline (p xliv), cover and examine The nuclei of the coloured corpuscles become stained of a deep red colour, the peri-nuclear part remaining unaffected, though of course the magenta has to traverse it to reach the nucleus All the corpuscles are not, however, equally affected In many the indications of the existence of an envelope with one or more thickenings in it are observable This last appearance is even better seen if the magenta be added after the blood has been irrigated with dilute alcohol

(*g*) **Tannic Acid.**—Mix a drop of the blood with a drop of a freshly prepared two per cent aqueous solution of tannic acid (made with hot water and allowed to cool) , cover and

examine The tannic acid acts on the serum-albumen of the plasma and forms a finely granular precipitate which somewhat obscures the field, but it also acts (like some other acids) on the corpuscles, causing them to become globular and the colouring matter to separate from the stroma The separated colouring matter tends to pass out of the corpuscle, but as it does so it becomes coagulated by the acid, and remains attached to the corpuscle in the form of one or more granular-looking buds (Pl I, Fig 5)

None of the preparations of the blood of the frog or newt hitherto described can be permanently preserved, the two following preparations may, however, be kept as permanent slides

(*h*) **Picric Acid and Picrocarmine**—Place a drop of blood on a slide, and add a drop of a saturated solution of picric acid, put the slide aside and allow it to remain for five minutes, and at the end of that time, when the acid has 'fixed' the corpuscles (that is, has coagulated their contents), the excess of acid should be removed by means of a narrow slip of blotting-paper A drop of a solution of picrocarmine (p xliii) should now be added, and a trace of glycerine to prevent evaporation, and the preparation set aside for an hour At the end of that time, remove the picrocarmine solution by means of a slip of blotting-paper, and add a drop of Farrant's solution or glycerine (p xlviii) and apply a cover The preparation may then be examined, when the peri-nuclear part of some of the corpuscles will be seen to be highly granular and of a deep yellow colour, while the nucleus is stained red In some of the corpuscles there may also be seen delicate yellow-coloured threads, extending from the nucleus to the envelopes In others the peri-nuclear part remains uniformly homogeneous

(*i*) **Osmic Acid and Picrocarmine**—Mix a drop of blood with a drop of a one per cent aqueous solution of osmic acid (p xlvi), and allow the slide to stand This 'fixes' the corpuscles without altering their shape At the end of five minutes remove the excess of osmic acid with blotting-paper, add a drop of solution of picrocarmine and a trace of glycerine to prevent evaporation, and set aside for three or four hours (or even longer, as no over-staining takes place) At the end of this time, the preparation is treated as (*h*) and examined The nucleus will now be found to be stained red, and the peri-nuclear part homogeneous and yellow If a drop of blood (taken from a frog which has been kept through the winter) be prepared by this method, some of the corpuscles may show 'vacuoles' in the peri-nuclear part (Pl I, Fig 7)

(*j*) **Osmic Acid and Logwood**—A similar preparation may be made by using a solution of logwood (p. xlii) as the staining agent instead of picrocarmine, with the advantage that the staining of the nucleus takes place much more rapidly—in about five minutes

(*k*) **Ammonium Chromate and Picrocarmine**—Mix a few drops of blood with a small quantity of a five per cent solution of ammonium chromate in a small glass vessel, and leave the mixture for twenty-four hours, taking care to prevent evaporation Then pour off the chromate solution and substitute picrocarmine solution for another twenty-four hours Mount a drop of the deposit in glycerine and examine it The effects of this substance are most remarkable Some corpuscles retain their shape though the nucleus enlarges, and shows an *intra-nuclear plexus of fibrils* In others the hæmoglobin is arranged in groups around the enlarged nucleus, while others have their wall partially dissolved on one side, with part of the cell-contents extruded through it.

The preparations (*i*) and (*j*) show colourless corpuscles with their nuclei stained

2 Observe the **Colourless Corpuscles** (H)—Prepare a drop of blood as for the examination of the coloured corpuscles On careful examination there may be seen, scattered amongst

the coloured corpuscles, colourless, irregular, granular bodies They are few in number compared with the red, and by careful searching three distinct varieties may be made out as follows —

(*a*) **Finely granular Corpuscles** —These have no definite shape, are colourless, and have a faint, finely granular appearance They are usually slightly smaller than the coloured corpuscles, and irregular in outline, with fine projecting processes Careful observation may discover nuclei, which are irregular in shape, and sometimes subdivided

(*β*) **Coarsely granular Corpuscles** —These also are indefinite in shape and colourless, but contain a large number of highly refracted granules, usually lying at one side of the corpuscle, whilst the subdivided nucleus occupies the other side They may be either larger or smaller than (*a*) and their processes are usually blunt and rounded

(*γ*) The third variety consists of small nucleated masses of protoplasm about the size of the nucleus of a coloured corpuscle , and as these latter sometimes escape from the corpuscles, care must be taken not to confound the one with the other

Amœboid movements of Colourless Corpuscles —The finely granular corpuscles (*a*) may be seen to change their shape and place (in the newt even at the ordinary temperature of the air) in the same manner as an amœba does, hence the term amœboid , and the coarsely granular corpuscles (*β*) behave in a similar manner In these latter, the granules, being more distinct, may be seen as it were to rush from one part of the corpuscle to another In this change the granules are passive, they are carried by the protoplasm The small nucleated masses (*γ*) undergo similar changes of site and form, but, on account of their smallness, these phenomena are not so easily observable The best way to notice these changes is to make drawings from time to time—say every two minutes—of a colourless corpuscle The change of form is sometimes so great that a corpuscle may actually be seen to divide into two At other times a transparent spot—a *vacuole*—which contains fluid may be seen in some corpuscles All these changes take place more rapidly when the slide is warmed on a hot stage (p xxviii) , and when this is done, it is necessary, in order to prevent evaporation, to surround the cover with a little oil or, better, with a ring of melted paraffin (*Sketch the changes of shape in* Pl I , Fig 8)

Feeding Colourless Blood-corpuscles —That the colourless corpuscles can take small particles into their interior is easily demonstrated, thus all that is required is to rub up a little china ink or vermilion in a three quarter per cent salt solution, and to mix a drop of this with the blood and examine the preparation from time to time on a hot stage, as indicated above The preparation requires to be sealed up with oil to prevent evaporation For newt's blood 30° C is sufficient

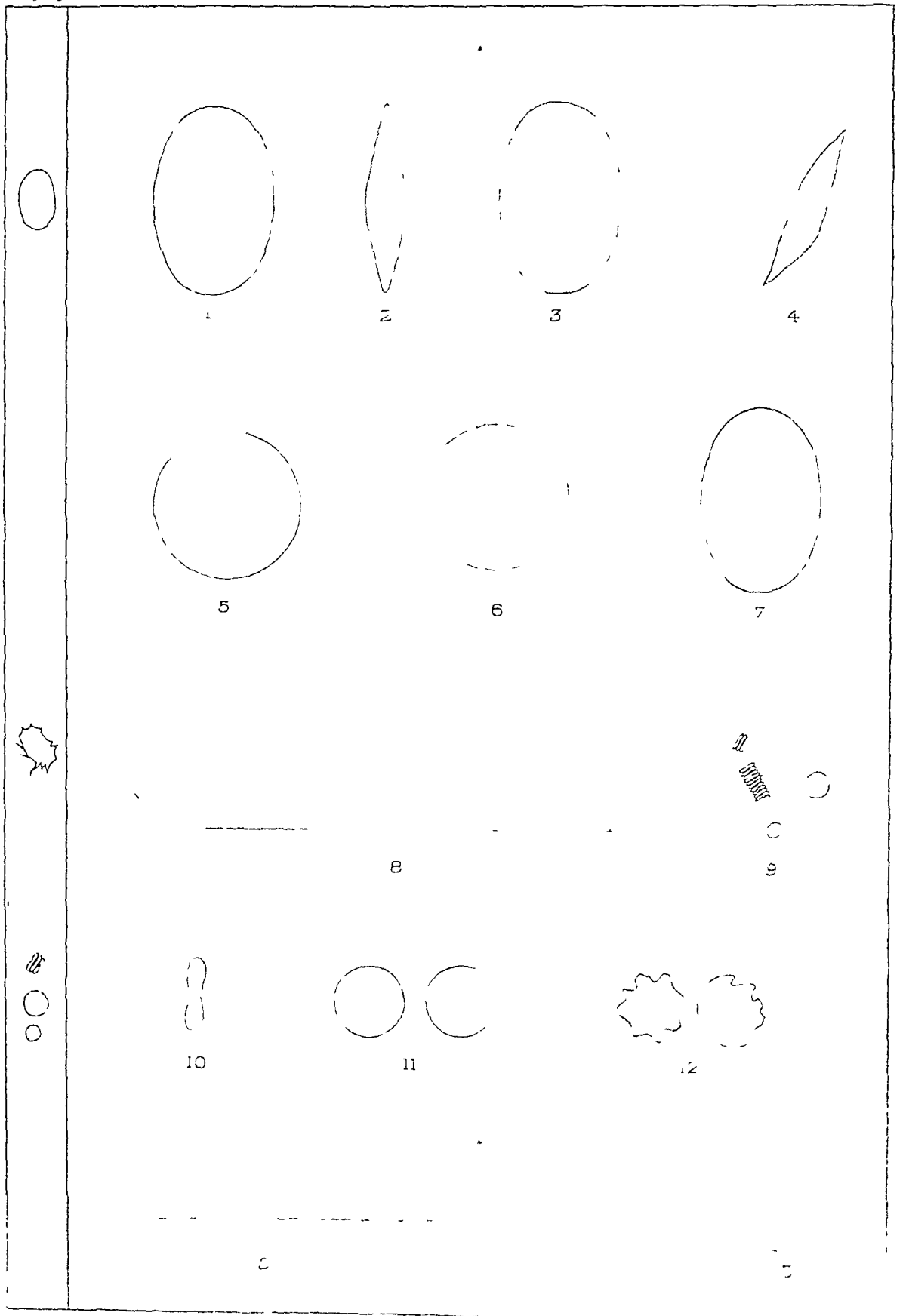
The **EFFECTS OF REAGENTS** on the colourless corpuscles should next be observed

(*a*) **Water** —When the blood is irrigated with water, the protoplasm of the white corpuscles swells up, becomes quite clear and transparent, and the nuclei, usually two or three in number, come clearly into view Sometimes also when so treated the granules may be seen to exhibit Brownian movement

(*b*) **Acetic Acid** —When blood is irrigated with a one per cent dilution of acetic acid, the protoplasm becomes clear and transparent, and a bi- or tri-partite nucleus is distinctly seen (*Indicate this effect in one of the corpuscles sketched in* Pl I , Fig 8)

(*c*) **Magenta** —When the blood is irrigated with magenta solution (p xliv), the protoplasm takes on a faint red, the nuclei a deep red colour (*Indicate the effects in one of the corpuscles sketched in* Pl I , Fig 8)

PLATE 1 BLOOD



Note—The osmic acid and picrocarmine, and osmic acid and logwood preparations of blood are the only permanent preparations which show colourless corpuscles A careful search is sure to reveal several corpuscles with their nuclei stained

In fresh blood an eighth-of-an-inch lens shows the intra-nuclear plexus of fibrils in the colourless corpuscles

(B) HUMAN BLOOD

PREPARATION—Wrap a handkerchief tightly round a finger, beginning at the base and proceeding towards the point, which will cause the point of the finger to become congested, then prick the skin at the root of the nail with a clean sewing needle and bring a slide in contact with the drop of blood which exudes Apply a cover and examine

EXAMINATION—1 Observe the **coloured corpuscles** (H) At first the coloured blood-corpuscles will be seen adhering by their flat surfaces so as to form *rouleaux* These piles may form a kind of network (*Indicate these rouleaux in* Pl I, Fig 9) When so disposed it is of course their edges only that are seen, but if the cover be gently moved with a needle-point, the *rouleaux* break up, and then the individual corpuscles are seen both on the flat and on edge On the flat they appear as bi-concave, circular discs, while edge-wise they appear somewhat dumbbell-shaped (Pl I, Fig 10) On carefully focussing a corpuscle as seen on the flat, a dark part at first appears in the centre, the margin being light (Pl I, Fig 11), but on altering the fine adjustment the centre becomes light, and the circumference dark (Pl I, Fig 11) These changes in appearance are due to the fact that the corpuscle is a bi-concave disc It is *not* due to the presence of a nucleus, for these corpuscles are non-nucleated and homogeneous throughout It is well to become familiar with the size of a corpuscle seen under a power of 300 diameters, for a corpuscle so magnified serves as a useful standard of comparison for other objects The diameter of a corpuscle is $\frac{1}{3500}$ inch It is generally stated that the coloured corpuscles are all of the same size, but this is not strictly correct, for a careful examination shows that there is a slight variation from this standard, and this variation is greater in some individuals than in others, and becomes specially marked in some diseases

EFFECTS OF REAGENTS (*a*) **Acetic Acid.**—When irrigated with one per cent dilution of acetic acid, the coloured corpuscles become globular and lose their colouring matter, which diffuses into the surrounding fluid, so that it is difficult to distinguish the outline of the individual corpuscles No nucleus is revealed

(*b*) **Water**—Irrigation with water decolourises the corpuscles and renders them globular The colouring matter is dissolved by the water which passes into the corpuscles by endosmosis and renders them globular The surrounding fluid becomes yellowish in colour

(*c*) **Tannic Acid**—If a drop of blood is mixed with a two per cent solution of tannic acid (p 2), one or more small refractive buds are seen to appear on the margins of the corpuscles These buds are produced by the same causes that produce similar buds on the corpuscles of the newt

(*d*) **Common Salt**—When blood is irrigated with a two per cent solution of common salt, *crenation* of the coloured corpuscles takes place—that is to say, they become jagged at the margins and on the surface, so that they resemble small thorn-apples At the same time, the corpuscles gradually become smaller and deepen in tint, but all the corpuscles are not affected to the same extent or with the same rapidity Perhaps this difference in the action of the

reagent is caused by some difference in the age or physical properties of the various corpuscles (*Indicate this in* Pl I, Fig 12)

(*e*) **Exposure to Air**—If a drop of blood be exposed to the air before the cover is applied, in some cases crenation occurs, but this phenomenon is not constant, nor when it takes place does it always occur to the same extent

None of these preparations of human blood make good permanent preparations

2 Observe the **colourless corpuscles** (H)—In a fresh drop of blood they are few in number—from five to eight being usually in the field at once They are best seen between the *rouleaux* of coloured corpuscles, and resemble in general characters the colourless corpuscles of the newt or frog They are, however, smaller and do not exhibit amœboid movements unless when kept at the temperature of the blood (38° C) on a hot stage (p xxviii) Some are larger than and others are about the same size as the coloured corpuscles, and for the most part they are all finely granular, though now and again a coarsely granular one is met with If the glass cover be touched with the point of a needle the colourless corpuscles are seen to adhere to the glass, unlike the coloured corpuscles, which freely roll over one another, and even when the coloured corpuscles impinge on a colourless one applied to the slide, they seldom displace it, so firmly do these adhere to any object (*Indicate the characters of the colourless corpuscles in* Pl I, Fig 13)

EFFECT OF REAGENTS—The actions of **Acetic Acid, Water** and **Magenta** on the colourless corpuscles of human blood are identical with the effects of the same reagents on the corresponding corpuscles of the blood of the newt or frog (*Indicate the effect of acetic acid in* Pl I, Fig 14, *and magenta in* Pl I, Fig 15)

3 **FREE GRANULES** (H)—Besides the two varieties of corpuscles just described (1 and 2) a number of very fine granules may be observed in the human blood

4 **FIBRIN** (H)—Place a cover on a drop of blood on a slide and allow it to stand for a quarter of an hour At the end of this time, on careful examination a number of delicate threads, which form an open network between the *rouleaux*, may be detected These threads consist of fibrin, and are still better seen if the blood be irrigated with a drop of magenta or iodine solution The former colours the threads red, the latter colours them yellow (*Indicate the appearance of the fibrin threads in* Pl I, Fig 9)

(C) BLOOD-CRYSTALS

5 **HÆMOGLOBIN**—The colouring matter of blood, hæmoglobin, may be obtained in a crystalline form, though it does not occur in that form inside the coloured corpuscles In the corpuscles it seems to lie in the meshes of a stroma

PREPARATION—Kill a *guinea-pig* and take a drop of blood from the heart or elsewhere, mix it on a slide with a drop of distilled water and cover The hæmoglobin diffuses from the blood-corpuscles into the water, and, as evaporation takes place, needle-shaped crystals form at the margin of the cover-glass They cannot, however, be preserved for any length of time, so as to make permanent preparations Or use the blood of a white *rat*, from which hæmoglobin crystals form in a few minutes

6 **HÆMIN**—Besides hæmoglobin, which exists normally in the coloured blood-corpuscles, and from which it may be obtained in a crystalline form, as above described, certain

other crystals—derivatives of hæmoglobin—may also be obtained from blood Of these (6), **Hæmin** (Hydrochlorate of Hæmatin), or **Teichmann's Crystals**, are of most importance, as their presence is considered one of the surest as well as one of the most delicate tests for blood in medico-legal cases

PREPARATION —Take a drop of blood from the finger and allow it to dry on a slide, or take some powdered dried blood previously prepared and add to it a trace of finely powdered common salt Moisten the whole with a drop of glacial acetic acid, apply a cover, and heat the slide gently over the flame of a spirit lamp till bubbles of gas appear Allow the preparation to cool, and apply a cover-glass

EXAMINATION (H)—Observe the short reddish-brown prismatic crystals scattered all over the field , they are best seen where they are attached to small masses of blood These are the crystals of Hæmin These make good permanent preparations To preserve them, take off the cover-glass, and remove the surplus acid with blotting paper Add a drop of Farrant's solution, re-apply the cover For the method of sealing up the preparation permanently, see p xlix

For the method of ascertaining the number of coloured blood-corpuscles by Malassez's method, see *Archives de Physiologie*, 1874, or the *Practitioner*, July 1878, where Dr Gowers describes the hæmacytometer The *amount* of hæmoglobin may be ascertained by means of the hæmaglobinometer See *Clinical Society's Transactions*, vol xii , p 64

EPITHELIUM

EPITHELIUM consists of corpuscles or cells with or without envelopes, of various shapes, sizes, and consistence, united by an intercellular substance or cement The corpuscles are always disposed on surfaces, and usually contain a nucleus and sometimes a nucleolus, they may be colourless, though they sometimes contain pigment They may be arranged in a single layer or in several layers Each cell regulates its own nutrition and never contains a blood-vessel The following is a convenient classification of the varieties of epithelium met with in the human body —(1) **Squamous,** (2) **Columnar,** (3) **Ciliated,** (4) **Transitional,** (5) **Secretory**

1 SQUAMOUS EPITHELIUM

PREPARATION —With the finger-nail or a blunt knife scrape off a little of the epithelium lining the inside of the cheek or covering the tongue Diffuse the scraping in water on a slide, cover and examine

EXAMINATION (H)—In this preparation only the **superficial squames** are seen They are irregular polygonal cells when seen on the flat, and are several times the breadth of a coloured blood-corpuscle Each cell contains a relatively small nucleus, and not unfrequently fine granules Occasionally several cells are found adhering to each other by their margins, and sometimes micrococci are seen attached to them If the edge of a cell be directed towards the observer, it will appear more or less spindle-shaped according to the angle which it forms with the plane of the slide (*Indicate these characters in* Pl II, Fig 1) In addition, small round and finely granular cells, about the size of a colourless blood-corpuscle and containing one or two nuclei, may be found here and there in the field These are the **salivary corpuscles** The included granules exhibit Brownian movement—a phenomenon which occurs when fine particles are suspended in a watery fluid, and which is probably due to differences of temperature in the several strata of the liquid Brownian movement is easily shown by rubbing down a little gamboge in water

EFFECT OF REAGENTS — Irrigate with a drop of magenta solution (p xliv) The nucleus stains of a deep red colour, while the peri-nuclear part is but slightly affected The alcohol in this solution precipitates the **mucin** of the saliva in the form of fine red threads or red membranous flakes (Pl II, Fig 1)

Squamous epithelium in exposed situations, as in the superficial layers of the skin, becomes hardened, and forms what is known as the **horny layer of the epidermis**

PREPARATION —With a knife shave off a few of the surface scales of the skin, diffuse the scraping in water, cover and examine

EXAMINATION (H)—The squames are flat, horny, and transparent, and usually no nucleus is visible

EFFECT OF REAGENTS—Irrigate with a five per cent dilution of liquor potassæ, the cells swell up and become globular Neither this nor the previous preparation need be preserved

INTRA-CELLULAR PLEXUS OF FIBRILS

It has recently been shown that the nucleus of cells contains a delicate plexus of fine fibrils—an **intra-nuclear plexus**—which is continuous with a similar plexus in the perinuclear protoplasm—the **intra-cellular plexus** It is this plexus, when the ends of its fibres are directed towards the observer, that gives the cells the appearance usually described as 'granular'

PREPARATION—Keep a newt in a small quantity of water for several days, but do not change the water At the end of this time the superficial layer of cutaneous epithelium will slough off in the form of a fine film Harden this film for twenty-four hours in a saturated solution of picric acid, or in absolute alcohol Preserve both in alcohol When required for use, snip off a small piece and stain it, if hardened in picric acid, with picrocarmine for half an hour, while the piece hardened in alcohol may also be stained with logwood Mount in Farrant's solution

EXAMINATION (H)—Observe the cells united by their margins Within each polygonal area notice the oval red-stained nucleus study it carefully, and a delicate plexus of fibrils—the intra-nuclear plexus—will be seen (Pl II, Fig 2)

Both in mucous membranes and in the skin, epithelium occurs in *several* layers, constituting stratified epithelium In order to see the deeper layers, and to study the relation of one layer to another, sections must be made A section of the conjunctival epithelium of the cornea is convenient for this purpose

Vertical section of the conjunctiva and cornea for **stratified epithelium**

PREPARATION—Remove the cornea from the eye of a cat just killed and place it in 40 c c of chromic acid and spirit mixture (p xxxi) consisting of two parts of a sixth per cent solution of chromic acid, and one part of methylated spirit Change the fluid at the end of the first, fourth, and seventh days, on the tenth day it will be sufficiently hardened For preservation transfer it to methylated or rectified spirit, after washing away all the surplus chromic acid When sections are wanted, put the cornea in a large glass of water for twenty-four hours to remove the spirit Transfer it to a solution of gum (p xxxviii) for twenty-four hours and then cut vertical sections in a freezing microtome (p xxxviii) After the sections are made, they are placed in a large quantity of water for twenty-four hours, to get rid of the gum, when they may be put into the preservative glycerine fluid (p xl) till they are required for mounting

EXAMINATION (H)—Float a section on to a slide (p xxvi), add a drop of solution of picrocarmine (p xliii), and allow it to remain on the section for fifteen minutes, or until the section is sufficiently stained Soak up the surplus staining-fluid with blotting-paper, and add a drop of Farrant's solution Cover and examine The fibrous cornea is stained red, but neglecting this, observe the layers of epithelial cells, stained yellow and their nuclei red, covering its anterior surface The deepest layer consists of cells columnar in shape, those on the surface appear as little more than lines or very elongated spindles, whilst between these extremes there are several layers of cells which show intermediate forms The cells are developed from below—from the lowest layer—and are gradually pushed upwards, and hence the change of shape (*Indicate these characters in* Pl II, Fig 5)

2 COLUMNAR EPITHELIUM

A FRESH COLUMNAR EPITHELIUM

PREPARATION—Slit open the small intestine of a cat just killed, and wash away the mucus covering its inner surface with a stream of ¾ per cent solution of common salt, scrape the mucous surface and diffuse the scraping in the same strength of salt solution, and after breaking it up with needles, cover and irrigate with magenta solution (p xliv)

EXAMINATION (H)—A large number of elongated, narrow, or columnar cells are to be seen Each cell is finely granular and contains a nucleus which is stained red One end of the cell—the attached end—is somewhat pointed, while the free end presents a narrow, clear, unstained hem, or border, marked with fine vertical lines or striæ This indicates that the free end of each cell is covered by a transparent plate or disc When several cells adhere to each other, and their free ends, instead of their sides, are presented to the observer, a fine mosaic is observed The intercellular substance is small in amount, mapping out the individual cells one from another, while in the centre of each area a red nucleus is seen (Pl II, Fig 4) In addition, here and there so-called '**chalice**' or '**goblet**' cells are seen (Pl II, Fig 6) These have a cup-shaped appearance, are devoid of the clear border and contain a nucleus surrounded by a small quantity of protoplasm near the lower pointed extremity of the cell, whilst the greater part of the body appears to be clear and looks as if it were empty This is not to be preserved, but, in order to obtain permanent preparations, the small intestine may be treated in one or other of the following ways

B PERMANENT PREPARATIONS

PREPARATION (*a*)—Place a square half-inch of the intestine in 40 to 60 cubic centimètres of a one per cent solution of ammonium bichromate for two days

(*b*) A similar piece in dilute alcohol (1 rectified spirit to 2 water) for twenty-four hours

(*c*) A similar piece in a few c c (5–10) of a one per cent solution of osmic acid (p xlvi) for half an hour

Any of these may be used for preservation, but in every case the specimen must first be steeped in water for half an hour or longer, to get rid of the hardening reagent

EXAMINATION (H)—Scrape off a little of the mucous surface of *a* or *b*, place it on a slide, and break it up with needles Add a drop of picrocarmine, and allow it to stain for fifteen minutes Remove the surplus staining-fluid, add a drop of glycerine, cover and examine

In either case columnar and chalice cells similar to those already described will be found, except that in *a* each cell will be yellow with a red nucleus, while in *b* the red nucleus will be seen to contain what has been described as a nucleolus If *c* be similarly treated, it must be left in the picrocarmine for twenty-four hours, when the same appearances are seen as in *a*, with this addition—that if the cells contain any fatty particles, these are blackened These three preparations when sealed as described (p xlix) are permanent

Columnar Epithelium from the small intestine of a newt—Where the cells are relatively large, and show very distinctly the intra-cellular and intra-nuclear plexus

PREPARATION—Kill a newt and place its small intestine in a few c c of a five per cent

solution of ammonium chromate for forty-eight hours After washing, transfer it to picro-carmine for twenty-four hours Open the gut, and with a knife scrape off a little of the epithelium and diffuse it in a drop of glycerine on a slide, cover and examine

EXAMINATION (H) —Observe the tall, relatively large, columnar cells, with a fine striated disc on their free ends, and a large red-stained nucleus A careful examination reveals the plexus of fibrils Besides these cells, look for 'goblet' or 'chalice' cells, which are somewhat cup-shaped, with a nucleus situated near the narrow, often tailed, end of the cell, and surrounded by a small quantity of protoplasm, while the upper part of the cell appears empty These cells secrete mucin, so that they may be regarded as unicellular glands The mucin seems to be derived from the interfibrillar substance (Pl II, Fig 7)

3 CILIATED EPITHELIUM

A LIVING CILIATED EPITHELIUM AND CILIARY MOTION

PREPARATION —Kill a frog by giving it a sharp blow on the head, but do not give chloroform, for it paralyses ciliary motion Scrape the mucous membrane of the roof of the mouth or gullet, and diffuse the scraping by means of two fine needles in $\frac{3}{4}$ per cent salt solution Place a hair in the fluid to prevent the cells being crushed, cover and examine

EXAMINATION (H) —Observe the groups of short columnar or nearly spherical cells with very granular contents, and having on one end vibratile cilia in active movement This movement causes currents in the surrounding fluid, which carry along with them any suspended particles, such as granules or blood-corpuscles Sometimes a detached cell may be observed to spin round and round by the motion of its own cilia The cilia themselves—which are fine homogeneous projections—are best seen when their movements become languid

EFFECT OF REAGENTS —Irrigate with a drop of magenta solution, and note that the colouring-matter does not stain the cells until the cilia have ceased to move Thus each cell *while living* can regulate its own nutrition There is a marked difference, therefore, in respect to colouring-matters between a living and a dead cell

B CILIARY MOVEMENT

For the study of '*ciliary movement*' the cilia of the common salt-water mussel are best adapted On opening the mussel with a knife, the yellowish-coloured gills are seen, and if a small part be snipped off and placed in the salt water found in the inside of the shell, teased with needles, covered and examined with a low power—(L)—the gills are seen as a series of bars fringed with cilia, much larger than those of the frog's epithelium (H) Observe the long, tapering, clear, and structureless cilia bending most at their tips Seal up this preparation by running a ring of oil round the margin of the cover-glass, and set it aside for several hours till the movement of the cilia becomes more languid (Pl II, Fig 10) The oil prevents the access of fresh oxygen, which is necessary for the ciliary motion, and as the oxygen in the fluid becomes used up, the ciliary action becomes slower Place the slide on a hot stage (p xxviii) and gradually raise the temperature to not higher than 35° C As the temperature rises, the ciliary movement becomes more rapid, but if the temperature be too high, so as to coagulate the albumen, a permanent arrest of the movement results An opposite effect is produced by the action of chloroform or ether Place a fresh piece of the gill on a cover-glass

in a drop of salt-water, and invert it over a gas chamber so that the gill is directed towards the chamber (p xxix)

EXAMINATION (L) —Ascertain that the cilia are active, then raise the cover-glass, put a small drop of chloroform into the chamber by means of a glass rod, and again apply the cover-glass The ciliary movement becomes slower and slower, and finally ceases If, however, the gill is removed before the chloroform has acted too long, and is freely exposed to air, the cilia may resume their movements (Lister) None of these specimens can be preserved

C PERMANENT PREPARATIONS OF CILIATED EPITHELIUM

PREPARATION —Place a small piece of the trachea of a cat, rabbit, ox, or sheep in dilute alcohol (1 to 2 water) for forty-eight hours, when the inter-cellular substance of the epithelium will be so softened that the cells can be easily isolated Then wash the whole piece in water, scrape the mucous membrane with a knife, place the scraping in a tube with a few drops of a one per cent solution of osmic acid The isolated cells are thus 'fixed,' and can be examined either with or without the addition of various staining reagents

EXAMINATION (H) —Place some of the cells in *picrocarmine* for at least twenty-four hours, transfer some to a drop of glycerine on a slide, cover and examine Observe the long, tapering character of the cells, with one end covered by a fringe of fine processes— the cilia—which are planted on a clear disc which is not stained The cell-protoplasm is described as granular, but careful examination shows that it contains an intra-cellular plexus of fibrils with the fibrils arranged chiefly in the long axis of the cell It is maintained by some observers that the cilia are actually prolonged through the clear band, so as to become continuous with this plexus The oval nucleus is placed far down in the cell, is bright red, and shows a similar plexus, with a bright spot in its centre—the so-called nucleolus The other end of the cell is often tapered or bifurcated (Pl II, Fig 8) Instead of the picrocarmine *logwood* may be used, with the advantage that a few minutes suffice for the staining process Either of these preparations may be sealed up and preserved permanently The ends of the cells may be directed towards the observer (Pl II, Fig 9)

4 TRANSITIONAL EPITHELIUM

PREPARATION —Take the bladder of a sheep, cat, rabbit, or guinea-pig, and prepare and preserve it in the same way as is directed for the preservation of ciliated epithelium

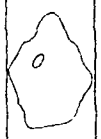
EXAMINATION (H) —Observe the great variety in the shape of the cells, some of them being more or less cubical, others resemble squames in character, while others again present a number of sharp angular points (Pl II, Fig 11)

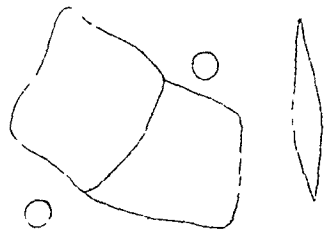
5 SECRETORY OR GLANDULAR EPITHELIUM

PREPARATION —Make a cut into the liver of a dog just killed, scrape the cut surface with a knife, so as to detach some of the liver-cells Place these in a few c c of a half per cent solution of osmic acid for an hour, then pour off the acid and substitute picrocarmine for several hours

x 300

PLATE II EPITHELIUM





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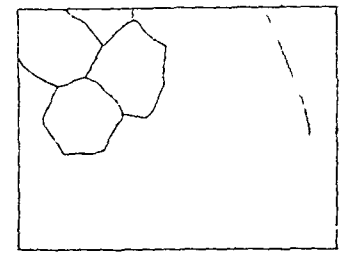

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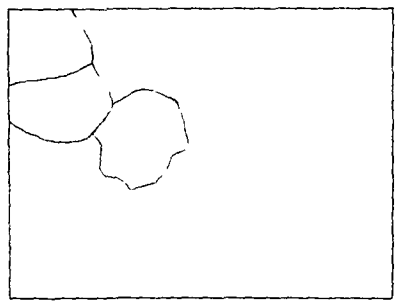

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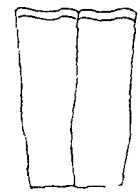

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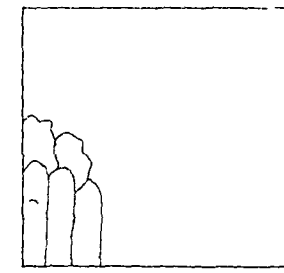

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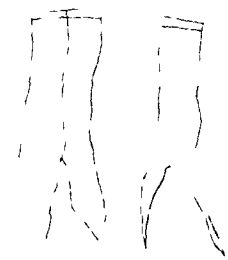

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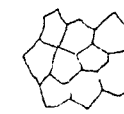

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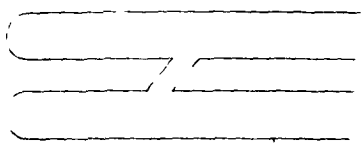

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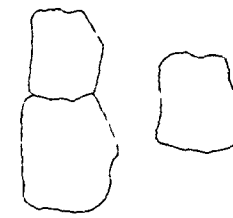

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EXAMINATION (H)—Many blood-corpuscles will be seen Neglecting these, study a liver-cell Notice its polygonal shape, yellowish granular protoplasm, and its nucleus deeply stained red No cell-envelope is visible Sometimes there are fine granules of pigment in the protoplasm, at other times fatty granules, especially if the liver of a stall-fed ox be used In young animals (dog) the liver-cells often contain two nuclei The 'granular' character of the nucleus and protoplasm are due to the existence of a plexus of fibrils, which requires a higher magnifying power to make it obvious (Pl II, Fig 12)

The liver-cells of the newt are very large, and are easily prepared by placing small pieces of the liver in a five per cent solution of ammonium chromate, and after washing, staining them with picrocarmine for several hours When teased out in glycerine they show most admirably the intra-nuclear plexus of fibrils The liver of the newt, especially at the end of winter, often contains particles of pigment (Pl II, Fig 12)

ENDOTHELIUM

It is convenient to apply this term to a single layer of squamous epithelium wherever it occurs, as the lining of serous sacs, blood- and lymph-vessels, &c

PREPARATION BY THE SILVER PROCESS—Bleed a rabbit, take a portion of its omentum and rinse it gently in distilled water to remove soluble chlorides Place it in a quarter per cent. solution of nitrate of silver for ten minutes or until it assumes a milky appearance Wash it thoroughly in ordinary water, and expose it to the action of diffuse sunlight, either in water or in water and alcohol, until it assumes a brownish colour Mount a small piece unstained in Farrant's solution, and to compare with it, stain a similar piece with logwood for ten minutes, and after washing away all the surplus logwood, mount it also in Farrant's solution Picrocarmine does not stain so well after silver, but after a tissue has been in alcohol for some time it takes up this pigment more easily The omentum of the rabbit is chosen because in it the endothelium occurs in a nearly continuous sheet

EXAMINATION (H)—The tissue will be mapped out into a series of small polygonal areas by narrow black lines—'silver lines' These are produced by a deposit of the reduced silver in the intercellular cement The flattened epithelial cells adhere by their margins and form a complete epithelium or endothelial investment for the connective tissue of which the omentum consists Focus carefully through the thickness of the membrane, and a second set of lines, whose outlines do not correspond with those lying above them, will be brought into view, these are the endothelial cells on the other surface of the membrane (Pl II, Fig 3) It is important to practise this method of focussing through the whole thickness of an object Usually no nucleus is observable within the cells, but in the logwood preparation each cell will be seen to contain an oval nucleus Preparations of other serous membranes will be made later on

CARTILAGE

In every cartilage there fall to be considered (*a*) the *matrix*, and (*b*) the *cartilage-cells* or *corpuscles* Cartilages are classified according to the nature of the matrix If the matrix be clear and like ground-glass it is called *hyaline cartilage*, if fibrous it is a *fibro-cartilage*, of which there are two varieties one, where the matrix resembles yellow elastic tissue, is *yellow fibro-cartilage*, and the other, in which the fibres resemble white fibrous tissue, is *white fibro-cartilage* Where the cells are separated by a very small quantity of matrix, the cartilage is said to be *cellular*, as in the ear of a mouse or in the chorda dorsalis

HYALINE CARTILAGE

Occurs in the articular ends of bones, cartilages of the ribs, part of the sternum, larynx, trachea and bronchi, and some nasal cartilages

A **FRESH HYALINE CARTILAGE** —Open any joint of an animal just killed, or take the cartilage of a rib from the same animal and make a thin slice of the cartilage with a razor Place the thin slice in a drop of blood-serum or one per cent alum solution, cover and examine Instead of making a slice, a piece of cartilage sufficiently thin may be obtained from the sternum of a newt or the tracheo-laryngeal cartilages of a frog

EXAMINATION (H) —If a thin slice has been made, observe the hyaline matrix dotted over with corpuscles, or cells—the *cartilage cells* Study first the cartilage-corpuscles and then the matrix The *corpuscles* are oval or of various shapes, granular and containing a large nucleus The protoplasm of the cells fills the entire space or lacuna in which it lies, but —especially near the margin of the section—the protoplasm may have fallen out of the lacunæ, or shrunk up so that it no longer completely fills the space in which it lies By focussing the cells are seen to lie in several planes The *matrix* is hyaline and perfectly homogeneous in its appearance (Pl III, Fig 1)

EFFECTS OF REAGENTS (*a*) **Water** —Irrigate the section with distilled water The protoplasm of the cell now shrinks rapidly from the cavity containing it It becomes coarsely granular and thus often obscures the nucleus

(*b*) **Iodine Solution** —Make a transverse section of a costal cartilage of a young animal Stain with iodine solution for three to five minutes, add glycerine and examine (H) This stains the matrix yellow, and the corpuscles of a deeper tint Within the protoplasm a brownish coloration may result, which indicates the presence of glycogen, this is best seen in young growing cartilage

B **METHODS OF PREPARING HYALINE CARTILAGE FOR PERMANENT PREPARATIONS** —It is necessary to use reagents to 'fix' the structural elements of the cartilage, and

amongst the best of these is a saturated watery solution of picric acid Take costal and articular cartilage as types

Place small pieces—not larger than half an inch—of the costal cartilages, with their perichondrium, of a cat or rabbit or human fœtus, in a saturated watery solution of picric acid for forty-eight hours Then remove them and wash them thoroughly in water till no yellow colour is given off, and place them in rectified spirit till they are required Sections may be made either with the hand or by means of a freezing microtome (p xxxviii) It is well to practise making sections with a razor, and cartilage, from its consistence, is one of the easiest tissues to begin with A similar procedure is adopted for *adult human costal cartilage*, and also for *articular cartilage* If the costal cartilage contain any bony or calcareous matter, macerate it for one or two weeks in the acid till all the calcareous matter is removed For *articular cartilage*, if it be desired to have a section of the subjacent bone, the bone must be softened by means of an acid which removes the calcareous matter It may be accomplished thus —Place the articular end of, say, the femur or humerus of a recently killed cat or rabbit in a large quantity (twenty to thirty times the bulk of the tissue) of one of the following solutions —

(*a*) **A saturated watery solution of Picric Acid** —Change the fluid at the end of a week and add fresh solution containing a few crystals of picric acid The bone will be softened in from two to three weeks

(*b*) **A quarter per cent solution of Chromic Acid** may be employed Take care to change the fluid after three days This fluid requires also from two to three weeks to decalcify the bone

(*c*) **A mixture of Chromic Acid and Nitric Acid** (p xxxiii) may be employed Similar conditions obtain as in (*a*) and (*b*)

(*d*) Or a two per cent **solution of Hydrochloric Acid** may be used as the decalcifying agent

(*a*) and (*c*) are the methods which yield the best results

COSTAL CARTILAGE

Make a number of transverse sections of the costal cartilage of a kitten or young rabbit, and also of the decalcified costal cartilages of an old person, prepared as described above These sections may be examined as they are or after being stained

A FROM A YOUNG ANIMAL

(*a*) **Osmic Acid** —Place some of the sections of the kitten's cartilage in a one per cent solution of osmic acid for twelve hours Wash them thoroughly in water, to remove the osmic acid Mount one in Farrant's solution, and cover These sections are taken, first, because they are small and one sees better the general characters of the section

EXAMINATION (L) —Observe the fibrous perichondrium and the cartilage-matrix inside it, the cartilage-corpuscles, small and flattened, lying in several layers Nearer the centre of the section are seen, as small specks, the irregularly shaped corpuscles The corpuscles are stained of a deeper yellow than the matrix by the osmic acid, hence its value (H) Examine the fibrous perichondrium with its areolar tissue and sections of elastic fibres Observe the cartilage-cells flattened towards the periphery of the cartilage, and the more rounded character of the cells placed nearer the centre Otherwise the cells and matrix have the same characters as are indicated in fresh hyaline cartilage

(*b*) **Carmine** —Stain a section with carmine Lay a section on a slide and on it place a

drop of solution of strong carmine (p xliii) Within a few minutes it will be stained of a uniform deep red colour Wash away the surplus carmine, and if the preparation is now examined (L) it will be of a nearly uniform red colour, the matrix scarcely distinguishable from the corpuscles in the depth of the tint Place on the section a large drop of a five per cent solution of glacial acetic acid for several minutes, examining the section with a low power all the time The effect of the acid is to remove the surplus carmine from the matrix and leave the corpuscles deeply stained red As soon as this is accomplished wash the section thoroughly in water, and mount it in Farrant's solution Strong glacial acetic acid accomplishes the same result more rapidly

EXAMINATION (H) —The general characters of the section are the same as (*a*), only the perichondrium and corpuscles are red and the matrix is colourless or only faintly stained (Pl III, Fig 2)

B FROM AN OLD PERSON

Make a number of transverse sections of the macerated tissue (p 15) Let the sections include a piece which has been calcified

(*a*) **Osmic Acid** —Place some of the sections in a one per cent solution of osmic acid as for fœtal cartilage

EXAMINATION (L) —Observe the perichondrium and the general arrangement of the corpuscles, the corpuscles flattened at the periphery, in irregular clumps a little further in, and, as one examines towards the centre, the rows of cartilage-cells produced by transverse or oblique cleavage The matrix, hyaline at the periphery, but finely granular in other parts This is best brought out by slightly shading the mirror or using a small aperture of the diaphragm

(H) The **corpuscles**—The protoplasm is very apt to shrink within the capsules, and in it are to be seen several black spots These are small masses of oil which have been blackened by the osmic acid, which is an excellent reagent for detecting the existence of fatty particles in any tissue, and is therefore of great value In persons above middle age, oil-drops are frequently to be found both in the cartilage-cells of the tracheal rings and rib-cartilages The rows of cells are obvious

Matrix —Examine the granular part Between the rows of cells the matrix may be found to be distinctly 'fibrillated', the fibres lie mostly parallel one with another

It is well to mount a preparation of cartilage, showing the fibrillation of the matrix in a ten per cent solution of common salt, which prevents the swelling up of the fibrils

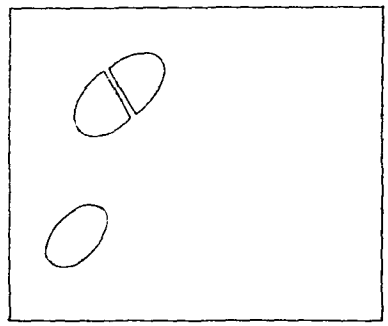
(*b*) **Eosin** —Place other sections in a dilute solution of eosin (p xlv) This substance stains the section very rapidly—a minute usually suffices Rinse them in a one per cent dilution of acetic acid and mount in Farrant's solution

EXAMINATION (L) —Observe a similar arrangement of the elements The matrix is slightly red and corpuscles slightly deeper in colour The calcareous matter deposited between the cells and the fibrillated parts are more deeply stained than the hyaline matrix, and a kind of stereoscopic effect can be given to these calcified parts by using a small aperture of the diaphragm

(H) Observe the highly refractive yellow oil-globules in the protoplasm of the cells, and observe particularly the more deeply stained cell-capsule or 'cartilage capsule' bounding the lacuna

(*c*) **Purpurine** —Place some sections in a solution of purpurine (p xliv) for forty-eight hours

PLATE III CARTILAGE



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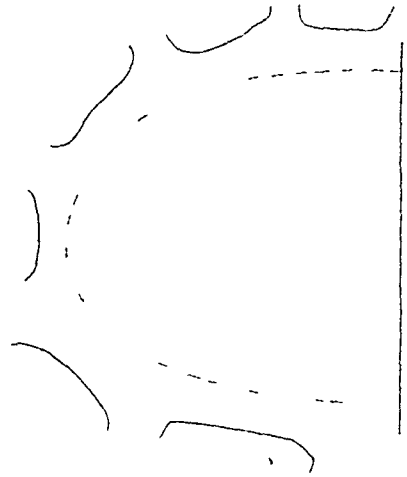

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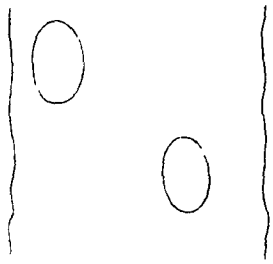

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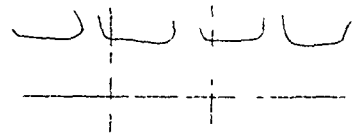

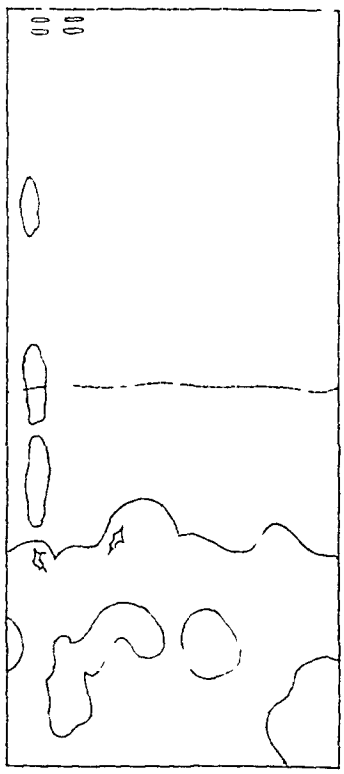

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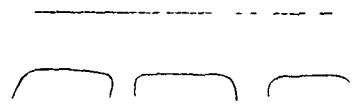

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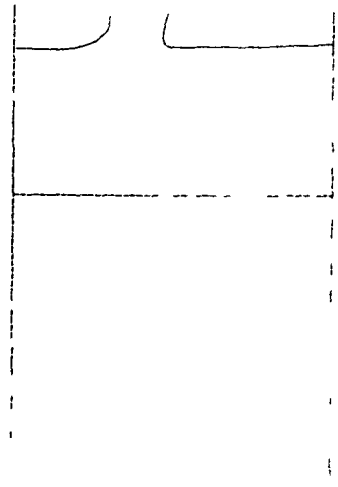


5

This substance stains the nuclei of the cells a light pink colour It possesses no advantages over the methods already indicated

The advanced student may study the action of chloride of gold and nitrate of silver on hyaline cartilage

Action of Chloride of Gold —Place in a one per cent solution of chloride of gold for half an hour *the articular end of the femur of a frog newly killed* Wash it thoroughly to remove the surplus gold, and place it in water acidulated with a few drops of acetic acid, and expose it to the light until the gold is reduced—usually for twenty hours—or until the cartilage becomes of a beautiful violet tint Make thin sections of the cartilage, and mount them in Farrant's solution

EXAMINATION (H)—Observe the cells arranged in several planes and stained of a deep violet colour They usually fill the lacunæ completely Often two cells may be seen together, in such relationship as to suggest that they have been produced by the division or 'fission' of a single cell The matrix is stained of a lighter colour

Action of Nitrate of Silver —Silver the articular cartilage of the femur of a freshly killed frog, as directed at p xlv, and after exposure to light, and when it has become brown, make a thin slice and mount it in Farrant's solution Cover

EXAMINATION (H)—Observe the matrix stained brown, but the cells are not stained, they are merely represented by oval or round clear spaces, which correspond exactly in shape and distribution with the cartilage-corpuscles The silver has not affected the cells, though it has darkened the intercellular substance The gold effect represents the positive, this the negative effect Staining with logwood reveals the presence of a cell in each apparently empty space

ARTICULAR CARTILAGE

Make a number of vertical sections of the head and subjacent bone of the femur or other long bone of a cat or other animal, decalcified according to the methods indicated (p 15), preferably by *a* or *c*

(*a*) **Osmic Acid** —Place sections in a one per cent solution of osmic acid for twenty-four hours Wash and mount in glycerine jelly (p xlviii), for Farrant's solution renders the section too transparent

EXAMINATION (L)—If the femur be chosen, observe the general arrangement of the parts from the articular surface downwards The superficial cells are flattened, and they always are parallel to the surface and at right angles to the axis of pressure A little below the surface they are in irregular clumps of twos and threes, and deeper down near the bone they are in rows, parallel to the long axis of the bone These rows are produced by transverse cleavage of the cells Below the cartilage is the bone The cartilage is divided into two zones, distinctly mapped off from each other by the character of the matrix Across the section, about two-thirds or half-way below the articular surface of the cartilage, there runs a line slightly wavy All that lies superficial to this has a *hyaline* matrix, and what lies below it, *i e* as far as the bone, is more or less granular and less transparent In this lower part the matrix is calcified This calcified part is distinctly mapped off from the subjacent bone, a well-marked wavy line with very distinct undulations—the one part dovetailing into the other, indicating

the line of demarcation If the section has been made from bone softened with chromic acid the bone will have a slightly greenish tinge

(H) Examine in detail the characters of the cells, and note especially the rows of cells in the deeper part of the cartilage These rows are produced by transverse cleavage of the cells One part of a row may lie in the hyaline matrix, while the other half is imbedded in the granular matrix (Pl III Fig 6) In some sections an indistinct fibrillation is seen in the lower part of the cartilage between the rows of cells, thus resembling what obtains in calcifying costal cartilage

(*b*) **Picrocarmine** —Put a section on a slide, and stain it with picrocarmine for twenty minutes to half an hour Remove all the picrocarmine, and mount it in glycerine jelly (p xlviii) The glycerine jelly must be melted in a water bath, and when fluid a drop is applied to the preparation by means of a glass rod, and covered at once, before the jelly sets

WHITE FIBRO-CARTILAGE

PREPARATION —Place a small piece of an intervertebral disc with its adherent bone, from a newly killed sheep or ox in twenty or thirty times its bulk of a mixture of chromic and nitric acid (p xxxiii) After two to three weeks the bone will be decalcified Wash the pieces in water to remove all acid, and preserve them in rectified spirit, or ,—

Remove the skin from the tail of a cat, cut it into pieces an inch long, and place these in chromic and nitric acid fluid

Make with a freezing microtome vertical sections of the intervertebral disc, including the subjacent bone, which will have a greenish colour Make also transverse sections of a piece of the decalcified tail of a cat or other animal

Place a vertical section of an **intervertebral disc** on a slide Stain it with strong *carmine* for ten minutes, or until it assumes a deep red colour Wash, and mount in Farrant's solution

EXAMINATION (L) —Observe the relation of the fibrous part of the disc to the subjacent bone

(H) The *matrix* in the ox is distinctly fibrous , the fibres are very fine, unbranched, and wavy The corpuscles are nucleated, and enclosed in a distinct capsule They lie scattered irregularly or in chains between the bundles of fibres Sometimes the cells are seen to be dividing Here and there fine dots, the cut ends of the fibres, may be seen (Pl III , Fig 3)

Stain with carmine a transverse section of an intervertebral disc from the already softened tail of a cat Remove the surplus staining fluid with blotting-paper, and mount in Farrant's solution

EXAMINATION (L) —Observe the concentric arrangement of the parts of the disc and the different directions of the fibres composing each layer of it

(H) Observe the fibrous character of the matrix and the corpuscles as before Here and there are transverse sections of the ends of the fibres, which appear like fine granules

YELLOW FIBRO-CARTILAGE

This may be easily obtained from the epiglottis, the arytenoid cartilages, or the external ear

PREPARATION—Place the arytenoid cartilages and the epiglottis of a sheep or ox (if the latter, cut it into two pieces) in a saturated solution of picric acid Remove it after forty-eight hours and make transverse sections of it in the usual way Preserve it in methylated spirit

Treat the external ear of a cat or rabbit in the same way and make sections This method is preferable to simple hardening in alcohol

Stain a transverse section of an epiglottis hardened as above with picrocarmine A few minutes suffice to stain the parts Remove the surplus staining fluid with blotting paper, and mount in Farrant's solution

EXAMINATION (L)—This specimen contains a section of the mucous membrane covering both surfaces of the epiglottis This may be neglected at present The cartilage itself is stained bright yellow (picric acid) with a perichondrium of connective tissue which is of a deep red colour Lying in the bright yellow fibrous matrix are a number of red spots, which are the nuclei of the cartilage-corpuscles

(H) Notice the *matrix*—yellow, distinctly fibrous, and the fibres like those of elastic tissue, and branched If the line of section cuts these fibres transversely they appear as fine yellow granules Trace these fibres into the red connective-tissue perichondrium, where they become yellow elastic fibres This continuity can be easily made out in a thin section Study the *corpuscles* Towards the periphery of the cartilage they are flattened, but near the centre they are rounded, have a distinct capsule, and the nucleus is distinctly stained red This forms an extremely beautiful preparation These preparations require a day or two for the colours to become differentiated

A section may be double-stained with picrocarmine and logwood, but this method possesses no advantages over the above

Stain a section of the ear of a cat with picrocarmine as above Mount it in Farrant's solution

EXAMINATION (L and H)—The yellow mass of cartilage stands out beautifully between the red connective tissue (Pl III, Figs 4 and 5) Sections of muscles, skin, and sebaceous glands may also be seen The same general characters are seen with a high power as are indicated above

TRANSITION FROM HYALINE TO YELLOW FIBRO-CARTILAGE

Make longitudinal sections of an arytenoid cartilage of an ox, and stain it with picrocarmine The upper half shows the structure of yellow fibro-cartilage, while the lower half is chiefly hyaline, with a few elastic fibres extending into the matrix This shows the mode of development of yellow fibro-cartilage from hyaline cartilage

CHANNELS IN THE MATRIX OF HYALINE CARTILAGE

These are easily seen in a section of a part of the cephalic cartilage of Loligo, which has been hardened in picric acid and stained with picrocarmine

CONNECTIVE TISSUE

CONNECTIVE TISSUE occurs in a variety of forms, as areolar tissue, or membranes, or forming fasciæ and tendons In whatever form it occurs we have to study two classes of structural elements, viz —*fibres* and *corpuscles* The fibres are of two kinds, (*a*) the *white* or *gelatigenous*, so called because they yield gelatine on boiling, (*b*) the *yellow* or *elastic*, which yield elastin The corpuscles are of three kinds (*a*) the *fixed connective-tissue corpuscles*, which always bear a definite relation to the white fibres, (*b*) *amœboid* or *wandering cells*, which are identical with colourless blood-corpuscles or leucocytes They wander freely in the lymph-spaces by virtue of their contractility, (*c*) the so-called *plasma* cells, of which the exact relation is not made out

ELASTIC TISSUE

A IN THE FRESH STATE

PREPARATION —Tear off, in the axis of the fibres, with a forceps, a very fine piece of the fresh ligamentum nuchæ of an ox Tease it out in a drop of salt solution or water, with two needles, and cover It is convenient to use a low power to ascertain when the piece is sufficiently teased

EXAMINATION (H) — Notice the homogeneous transparent fibres, about the breadth of a human coloured blood-corpuscle They have a yellowish tinge, and their margins are well-defined They branch and anastomose and may curl up at the ends (Pl IV, Fig 1)

EFFECT OF REAGENTS —Irrigate the preparation with a one per cent dilution of acetic acid The fibres are not affected thereby To preserve this preparation substitute Farrant's solution for the acid watery fluid

B PERMANENT PREPARATIONS

Excellent permanent preparations are obtained by the following method Place small pieces—about half-inch cubes—in many times their bulk of chromic and nitric acid fluid (p xxxiii) for a week After that soak the pieces in water for twenty-four hours, to remove all traces of the acid, and preserve them for use in methylated spirit Make longitudinal and transverse sections in the usual way

Stain a longitudinal and a transverse section in picrocarmine for ten minutes

EXAMINATION (H) of the longitudinal section reveals the elastic fibres stained of a deep yellow, with their characteristic branches and anastomoses Between them a small amount of ordinary connective tissue stained red is seen

The transverse section shows the cut ends of the fibres, as solid yellow polygonal bodies in

groups of twos and threes, when two fibres adhere their opposed surfaces are moulded to each other (Pl IV, Fig 2) Surrounding each group of fibres is a very small quantity of connective tissue stained red Only a very small quantity of white fibrous tissue exists in the ligamentum nuchæ If desired similar sections may be tinged with a solution of magenta (p xliv), which stains the elastic fibres bright red, and is thus a good reagent for detecting their presence

Elastic tissue also forms part of ordinary areolar tissue It occurs in the form of a *network of fine elastic fibres* in the skin, where it will be examined, but its characters may be well studied in the **mesocolon of a rabbit**

PREPARATION —Remove the mesocolon from a newly killed rabbit Pin it to a piece of flat cork to keep it stretched, and place it, cork uppermost, for three days in a mixture of chromic and nitric acid (p xxxiii) After washing away all surplus acid, preserve it in methylated spirit Snip off a small piece, float it in water to remove the spirit, and stain it for ten minutes with picrocarmine, mount it in Farrant's solution

EXAMINATION (H) —Observe the network of fine elastic fibres one-sixth the breadth of a coloured blood-corpuscle The fibres are imbedded in white fibrous tissue, which is stained red

If desired another piece may be stained with solution of magenta (p xliv) which stains the elastic network red Elastic tissue will be further considered with arteries, and in the skin, where the method of preparing it by artificial digestion is described (Stirling) (p 92)

WHITE FIBROUS TISSUE

IN THE FRESH STATE

PREPARATION —Kill a rabbit or a rat, and with scissors snip off a small piece of the delicate connective tissue that lies under the skin, and which connects it to the fasciæ The piece of tissue at once rolls into the form of a ball, but by placing it on a *dry* slide it can easily be spread out with needles into a fine membrane, its original form when *in situ* During the process do not allow the tissue to dry, which may be prevented by breathing on it from time to time Add a drop of normal salt solution, cover and examine

EXAMINATION (H) —Observe the wavy fibres of white fibrous tissue running in every direction Their outlines are indistinct, but here and there a longitudinal striation in their course is observable, indicating that each fibre is composed of *fibrils* In addition to these, a few fibres which refract the light more strongly may be detected, these are elastic fibres, but little else is observable in this preparation, especially if taken from an adult animal (Pl IV, Fig 3)

EFFECT OF REAGENTS (*a*) **Magenta Solution** —Irrigate the preparation with solution of magenta (p xliv), and the existence of the corpuscular element will be revealed The nuclei of the connective-tissue corpuscles are stained of a red colour by the dye There are two kinds of corpuscles, one larger than the other The larger, when seen on the flat, are like large nucleated squames, though they are sometimes branched, and when seen on edge they are fusiform These are the 'fixed' connective-tissue corpuscles, and the smaller ones, about the size of the colourless blood-corpuscles, with two or three small nuclei, are the wandering cells, or leucocytes, and are identical with lymph- or colourless blood-corpuscles The elastic fibres

—which are recognised by their branching and forming a network—are stained red, while the white fibres are but slightly acted on by the magenta This preparation need not be preserved The observer is not to suppose that the corpuscular elements do not exist before the addition of a reagent, they do exist, but, owing to their refractive index being so near that of the fluid in which they are examined, they are not visible until some staining reagent is added It is further to be remembered that the relation of the fixed corpuscles to the white fibres is changed in a teased preparation Occasionally large oval granular and nucleated cells are found, especially along the course of the vessels, they are the so-called *plasma cells*

(*b*) **Dilute Acetic Acid** —Make a similar preparation, and irrigate it with a one per cent dilution of acetic acid

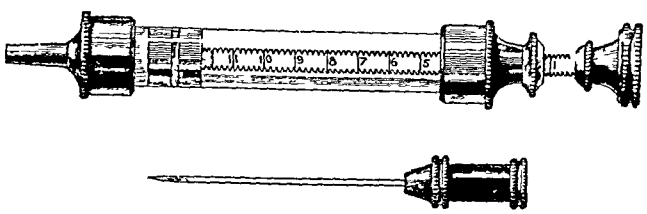
EXAMINATION (H)—Note that, as the acetic acid displaces the salt solution, it acts upon the white fibres and causes them to swell up, to become transparent, so that they appear quite homogeneous and jelly like, though they are not dissolved Some of the fibres may, however, present annular constrictions in their course This clearing up of the white fibres brings the other elements in the tissue more clearly into view—to wit, the elastic fibres and the corpuscles The elastic fibres are highly refractive, and their branches unite to form a network, and if such a fibre be broken across it curls up at its ends, though otherwise they pursue a straight course Numerous fusiform or oval nuclei are revealed, they are the nuclei of the fixed connective-tissue corpuscles, whilst the small bi- or tri-partite nuclei belong to the lymph-corpuscles The acetic acid may be displaced by magenta solution, when the elastic fibres and the nuclei are stained of a bright red colour (Pl IV, Fig 4) Do not preserve this

AREOLAR TISSUE

PERMANENT PREPARATIONS OF AREOLAR TISSUE

PREPARATION (*a*)—With a small subcutaneous syringe (fig 27) forcibly inject a quantity of a half per cent solution of osmic acid under the skin of the groin of a young dog, rat, or rabbit just killed, until an artificial œdema or bulla is formed After half an hour, snip open the bulla, taking care that no hairs get into it With scissors snip off a small piece of the distended connective tissue and place it on a slide Spread it out as a membrane with needles, and add picrocarmine It will require several hours to stain, and to avoid desiccation add a small drop of glycerine and cover When it is sufficiently stained, remove the picrocarmine, and substitute glycerine containing a trace of formic acid

FIG 27



Subcutaneous syringe for interstitial injections, and the cannula The hollow needle ought to be made of gold, to prevent acids from acting on it Natural size

(*b*) A similar preparation may be stained with logwood—which acts in a few minutes, remove the logwood solution and mount in glycerine

(*c*) Instead of injecting osmic acid, the subcutaneous injection of a saturated watery solution of picric acid does very well Stain the section with picrocarmine, as directed under (*b*)

EXAMINATION (H)—The corpuscles are now clearly brought into view, their nuclei are stained and their wing-shaped expansions are distinct,—in fact, they appear as flat, nucleated plates, sometimes with divided expansions Elastic fibres are yellow, and white fibres are slightly reddish

Annular Constrictions on the White Fibres—Take a small piece of the delicate pia mater lying within the circle of Willis from the brain of a sheep just killed, or slit up the dura mater of the spinal cord of an ox, and remove some of the fine fibres that stretch across the sub-arachnoid space Spread out the tissue on a slide, and irrigate it with a one per cent dilution of acetic acid The fibres swell up, but here and there they exhibit numerous annular constrictions These constrictions are the indications of the existence of a sheath on the fibres, which is not affected by acetic acid In some cases a fibre may be found to wind in a spiral manner round the white fibre These preparations may be stained with picrocarmine or magenta, but they do not keep well

The fibres of white fibrous tissue are made up of fibrils, held together by a cement—The fibrils ($\frac{1}{50000}$ inch in diameter) are sometimes revealed by teasing, but it is better to use some chemical reagent to dissolve the cement

PREPARATION—Place a narrow strip of the tendo Achillis or other tendon of a rabbit or other animal in a saturated solution of picric acid (p xxxii) for twenty-four hours After that time wash away the surplus colouring matter, tear off a small shred, and tease it in water Cover

EXAMINATION—The wavy, excessively delicate, non-branched, yellow-coloured fibrils (due to picric acid) are now apparent Each white fibre is made up of a great number of these fibrils, held together by a cement Usually no cells are seen, but irrigation with a one per cent dilution of acetic acid readily brings the long oval nuclei of the tendon-cells into view

OTHER METHODS—Maceration of a piece of tendon in a saturated watery solution of baryta for four or five hours, and subsequent steeping in water for twenty-four hours—or maceration in a large quantity of a ten per cent solution of common salt for ten days, effect a similar result, but the picric acid method is the most satisfactory and convenient

TENDON

It is advisable to use a tendon from a young animal, for in such there is a much larger number of corpuscles than in the tendon of an adult

PREPARATION—Place pieces of the tendo Achillis of a calf—about an inch long and a quarter of an inch thick—in many times their bulk of Muller's fluid (p xxxii) for fourteen days Then remove them, and after soaking them in water to remove the chromium salts, preserve them in rectified spirit till sections require to be made in the usual way by freezing Make both transverse and longitudinal sections

TRANSVERSE SECTION

(1) Place a section on a slide and cover it with a large drop of logwood solution, within a few minutes it will be sufficiently stained Wash away all the surplus logwood and mount the section in Farrant's solution

EXAMINATION (L)—Observe the *sheath* of the tendon, composed of connective-tissue fibres disposed circularly It sends processes into the substance of the tendon, subdividing it into polygonal areas These areas are made up of the cut ends of the longitudinally disposed fibres which constitute the tendon Each area has scattered in it a number of small specks deeply stained by the logwood, united by fine lines These are the spaces—the inter-fascicular lymph-canals—between the bundles of fibres, and in each space a corpuscle may be detected (Pl IV, Fig 6)

(H) The great mass of the tendon is but slightly stained, but notice the sections of the corpuscles, which are deeply tinted The corpuscles give off processes which may be traced a certain distance between the fibre-bundles Amongst the cut ends of the fibres may be seen small, light, refractive points, these are the ends of elastic fibres—few in number—which exist in tendon (Pl IV, Fig 7)

The branched *interfascicular* spaces in the transverse section are best seen in a *transverse section of a dried tendon* examined in water The spaces appear branched and black and larger than natural, from the shrinking of the bundles of fibres bounding them They are black because they contain air The addition of glycerine renders all the parts too transparent, hence such a preparation is not permanent

Sometimes the logwood stains the section of an almost uniform dark violet tint, and it becomes impossible to distinguish the corpuscles from the rest of the section Under these circumstances, irrigate the over-stained section with a five per cent dilution of glacial acetic acid, examining the section all the time with a low power The colouring matter will be dissolved out of the fibres and remain in the corpuscles, and if the acid is thoroughly washed out of the preparation, this may be mounted in Farrant's solution and preserved It is well to place such a section for a few minutes in a five per cent solution of bicarbonate of soda to remove all the acid This process of overstaining a section with either logwood or carmine, and getting rid of the excess by means of acetic acid, is frequently of value

LONGITUDINAL SECTION

(2) Place a section on a slide and stain it with logwood, as directed for a transverse section

EXAMINATION (H)—Observe the fibres arranged longitudinally, many of them slightly wavy, their outlines indistinct and always unbranched Between the fibres, which are but slightly stained or not at all, rows of elongated fusiform corpuscles deeply stained are observed Study these corpuscles They are nucleated and the nucleus is surrounded by a granular mass of protoplasm which forms part of the body of the corpuscle They are fixed connective-tissue corpuscles or 'tendon cells,' and are most numerous in young tendons (Pl IV, Fig 5)

(3) Tease out with needles a small piece of a section treated as (2) The fibres and corpuscles are easily isolated Notice the nucleated granular corpuscles in many cases adhering to and partly encircling a fibre These corpuscles when seen on edge are fusiform, but when detached and seen on the flat they are more or less flattened, with their edges sometimes turned in These cells partly surround and form an imperfect sheath for the fibres

Ranvier has shown that the relations of the cells to the fibres of a tendon can be more successfully made out in the fine tendons found in the tail of a young rat or mouse

x 300

PLATE IV CONNECTIVE TISSUE & TENDON

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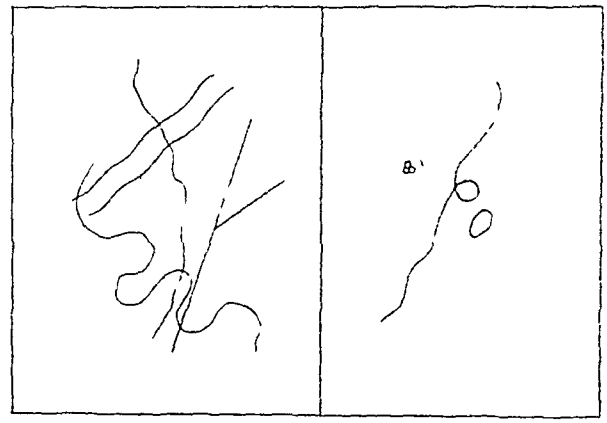

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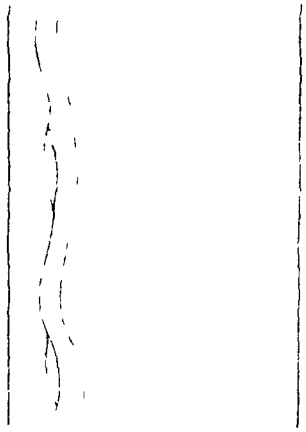

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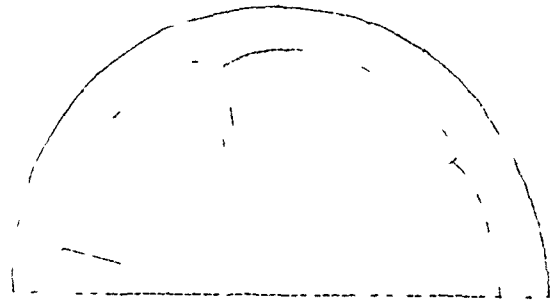

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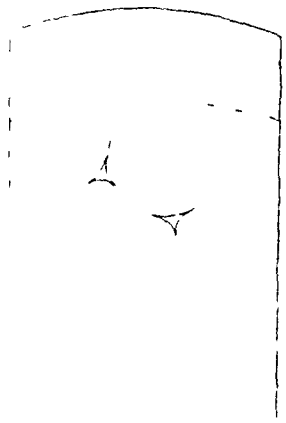

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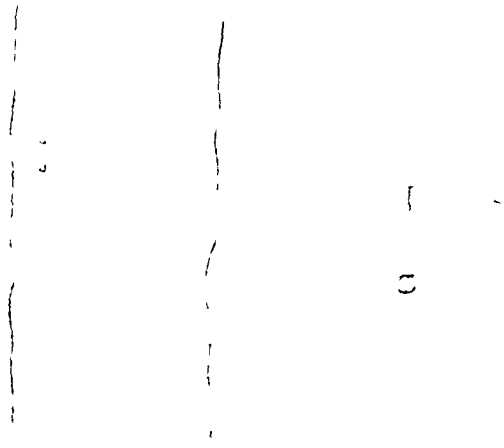

8

TENDONS IN THE TAIL OF A RAT OR MOUSE

PREPARATION—Kill a rat—preferably a young one—seize the tip of the tail, and forcibly pull it This ruptures the caudal vertebræ, and a leash of fine silvery-looking tendons is pulled out Place several of these tendons in the filtered juice of a lemon Leave them there for three minutes They become clear and transparent and swell up considerably Remove them, wash them thoroughly in water, and place them in several times their bulk of a one per cent watery solution of chloride of gold (p xlv), and allow them to remain there for fifteen or twenty minutes They become of a deep yellow colour, contract somewhat, and are rendered more rigid Remove them from the gold solution, and wash away the surplus gold in water Place them in an ounce of a twenty-five per cent watery solution of formic acid The bottle containing them must be kept in a dark place for twenty-four hours At the end of this time, the tendons are removed from the formic acid and are thoroughly washed in water They have now a decided purple colour throughout The formic acid reduces the gold on which it is deposited in the tissues, and to a greater extent in the protoplasm of the cells than in the fibres There are other ways of employing the 'gold method,' but we have found this modification of Ranvier's very successful [Each student cannot do the entire process, but he is shown how to do it] Take a small piece of one of the above tendons, and tease it with needles, in a drop of glycerine, and apply a cover-glass, or what does very well, gently press on the cover-glass until the tendon is flattened

EXAMINATION (H)—Observe the rows of flattened quadrilateral cells lying between the longitudinally disposed fibres The central part of each cell which contains the nucleus is of a dark purple colour and finely granular Thin slightly coloured wings may be seen extending outwards from this The position of the nucleus is often left unstained and is usually placed at one end of the cell, and the nuclei of adjacent cells in a row are usually so disposed that the two nuclei lie close together One or more dark lines may be seen running along the centre of the cells (Boll's stripe) These are ridges projecting in a direction vertical to the plane of the rectangular plate of the cells (Pl IV, Fig 8) It is easy, by teasing out the preparation carefully, to isolate a fibre with some of the cells still adhering to and partly embracing it, so as to indicate the relation of the cells to the fibres They can be seen to send their thin lamellar or wing-like expansions around the fibre, so as partly to embrace it (Pl IV, Fig 9) These cells partly occupy the branched interfascicular spaces seen in a transverse section of tendon (p 24)

DOUBLE-STAINING OF TENDONS

Gold Chloride and an aniline dye—Place an inch of the tail of a rat in gold chloride for an hour, and then treat it as above After the gold is reduced place the tail in chromic and nitric acid fluid, to remove the lime-salts (p xxxiii) Make transverse sections, and stain them with iodine green and aniline blue, and mount them in dammar (p l) Beautiful sections of tendons stained with gold are obtained, while part of the bone or interstitial cartilage becomes green These form most instructive preparations

CELL-SPACES IN TENDON

The above-described cells lie in spaces, which may be demonstrated by a solution of

nitrate of silver—just as in cartilage (p 17), the silver blackens the intercellular parts and leaves the spaces occupied by the cells clear

PREPARATION—Take several of the freshly exposed tendons of the tail of a rat Wash them with a camel-hair pencil dipped in distilled water Do this several times to remove the epithelium which covers their surface Place the tendons in a half per cent solution of nitrate of silver for five minutes Then wash them in water and mount a small piece of a tendon so treated in glycerine, and expose it to the light until it assumes a brownish colour

EXAMINATION (H)—Observe the irregular spaces seen in a dark brown matrix These spaces communicate one with another by fine clear lines The brown stained part indicates the existence of an intercellular substance, in the clear spaces lie the cells with their processes

EPITHELIAL COVERING OF TENDONS

Each of the fine tendons is enveloped by a layer of squamous epithelium, which is easily demonstrated by staining the tendons—*without previous pencilling*—with nitrate of silver as directed above Mount a tendon in glycerine, and expose it to light until it becomes brownish in colour

EXAMINATION (H)—The outlines of the polygonal epithelium are distinctly seen These indicate the existence of an epithelial investment of the tendon superficial to the fibres and cells already described

MEMBRANOUS CONNECTIVE TISSUE

THE OMENTUM

A convenient form of this tissue is found in the omentum, which differs in its characters in various animals, and also in young and in adult animals In an *adult cat* the omentum consists of bundles of connective tissue of various sizes, cemented to each other in the same plane, so as to form a network In the larger trabeculæ blood-vessels and fat-cells are found Each bundle of fibres is *completely* invested by a layer of squamous epithelium or endothelium, and the nuclei of these cells can readily be seen, on the surface or edges of the fibres when they are stained The trabeculæ consist of fibrils which can be seen in the fresh tissue In the substance of the trabeculæ and amongst the fibrils a few fusiform corpuscles—the connective-tissue corpuscles—may be seen They are relatively few in number, and so are the elastic fibres

In a *young growing rabbit* the omentum is much less fenestrated, and forms a more continuous sheet of connective tissue, covered on each surface with a layer of endothelial squames As the animal becomes older the membrane becomes more fenestrated

PREPARATION **Silver Nitrate**—Remove the omentum from an adult cat and rabbit, and also from a young rabbit just killed Wash each in distilled water to remove the soluble chlorides, and then place them in a quarter per cent solution of nitrate of silver for five minutes, and, after washing them thoroughly, expose them in alcohol to the light till they become brown Snip out a small piece of each with scissors, and float them from water on to slides in the usual way (p xxvi) Mount in Farrant's solution and cover, or similar preparations may be stained with logwood, which stains the nuclei

EXAMINATION (L and H)—Observe the nearly continuous layer of endothelium in the case of the young rabbit (Pl V, Fig 1) on each surface of the omentum (p 26), the more fenestrated character in the adult rabbit, and the almost net-like condition in the cat (Pl V, Fig 2) In all cases the silver lines, indicating the existence of the endothelium covering the trabeculæ, are seen Focus down through one of the larger trabeculæ, inside which connective-tissue corpuscles may be found Do not confound the large oval nuclei of the squames which are always on the surface with the nuclei of the connective-tissue corpuscles proper Omit at present the study of the blood-vessels and the fat cells which frequently accompany them

The omentum of a young rabbit is particularly valuable for studying the development of fat-cells and blood-vessels

ADIPOSE TISSUE

This consists of small vesicles or cells filled with fatty substances The cells are imbedded in a small quantity of areolar tissue containing blood-vessels Each cell has a distinct envelope, and contains a globule of clear, sometimes slightly yellow-coloured fat, which nearly fills the cell, so that the nucleus, with a small amount of protoplasm surrounding it, is pushed to one side and compressed against the cell-wall Study fully formed cells and fat-cells developing

PREPARATION—Snip off a small piece of fat and tease it in a drop of salt solution on a slide Place a piece of paper between the slide and cover-glass, to prevent the latter pressing on and rupturing the envelopes of the cells

EXAMINATION (L and H)—Observe the relatively large size ($\frac{1}{300} - \frac{1}{600}$) of the clear, highly refractive vesicles, sometimes compressed against each other Usually no nucleus is visible If the cells have been ruptured, globules of fat of various sizes will be found floating about Irrigate with magenta solution (p xliv), which stains the nuclei of the cells They are seen as small oval red spots compressed against the cell-envelope Substitute glycerine for the magenta, and mount for preservation (*Indicate this in* Pl V, Fig 4.)

Effect of Osmic Acid—A similar preparation, without the magenta, may be irrigated with a one per cent solution of osmic acid, which blackens fatty matter, and hence is a most valuable reagent for its detection

Examine the omentum of the cat (p 26) and the preparations of areolar tissue (p 22) for fat-cells

Crystals of Margarine—Delicate, needle-shaped crystals, springing from a point within the fat-cells, often occur They are post-mortem appearances—for the contents of fat-cells are fluid during life—and may readily be produced by steeping a morsel of fat for twenty-four hours in glycerine, or long steeping in alcohol, or after partial digestion (p 92)

The **Envelope of Fat Cells** is easily shown by steeping a morsel of fat in ether, which dissolves out the fat and leaves the empty shrivelled-up envelopes (*Indicate this in* Pl V, Fig 5)

DEVELOPMENT OF FAT-CELLS

PREPARATION—Place a small piece of the skin of a fœtus in rectified spirit for three days At the end of that time, make vertical sections in the ordinary way by freezing After the gum has been removed from them by steeping in water, place some of them in a

small quantity of a one per cent solution of osmic acid for five hours Wash them thoroughly in water, and mount one in Farrant's solution Cover

EXAMINATION (L)—Note the skin and the subcutaneous tissue, and, scattered throughout the latter, various sized groups of black spots These black spots are of various sizes, and are the fat-cells, which have been blackened by the osmic acid Select a group and examine (*Indicate this general arrangement in* Pl V, Fig 3)

(H) Notice the different sizes of the various vesicles composing each group Some of the vesicles appear to be nearly filled with a blackened globule of fat, whilst others contain only one or two small black granules of fat, the rest of the cell being occupied with a finely granular protoplasm These cells are connective-tissue corpuscles in process of being transformed into fat-cells through the degeneration of their protoplasm into fat Developing fat-cells may be well studied in the connective tissue of the infra-orbital region of a young rabbit

PIGMENT IN CONNECTIVE-TISSUE CORPUSCLES

Connective-tissue corpuscles containing pigment are seen in the skin of the frog or in its mesentery, and, in fact, in nearly all its tissues Preparations will be obtained from the choroid coat of the eyeball of any animal (p 107)

MUCOUS TISSUE

Mucous or embryonal tissue has a close relation to adipose tissue, as in the embryo it forms the subcutaneous tissue which ultimately becomes panniculus adiposus in the adult It is essentially an embryonal tissue, as in addition to the site mentioned above, it occurs only in the umbilical cord (Wharton's jelly), and in the vitreous humour of the eye Acetic acid precipitates the mucin in it in the form of fine threads It consists of fusiform and branching cells, whose processes communicate in all directions with each other, so as to form a meshwork, whose meshes are filled with a clear transparent mucin-yielding ground substance In it also are flat endothelial cells similar to those found in connective tissue It soon changes its character as the embryo grows older, white fibrous tissue appears in the matrix, so that it comes partially to resemble connective tissue, and then to pass into adipose tissue In the umbilical cord this last change does not take place it remains in the fœtus at full time as a fibrous tissue containing a mucin-yielding fluid in its meshes

PREPARATION (*a*) **Subcutaneous Mucous Tissue**—Inject into the axilla or groin of as young an embryo as it is possible to get a small quantity of a quarter per cent solution of osmic acid, so as to form a bulla Snip out a small part of the œdematous tissue, stain it with logwood or picrocarmine, and mount in glycerine

EXAMINATION (L and H)—Observe the fusiform and also the branched cells anastomosing with each other with their nuclei stained Use a very small aperture of the diaphragm

(*b*) **Umbilical Cord**—Harden part of the umbilical cord of a four months' fœtus in Muller's fluid for a week, and make transverse sections Stain a section with logwood, and mount it in Farrant's solution Another may be stained with methyl-aniline (p xliv) and mounted in a saturated solution of acetate of potash

EXAMINATION (L and H)—Observe the three umbilical vessels (p 124) surrounded by

PLATE V OMENTUM FAT &c

× 300

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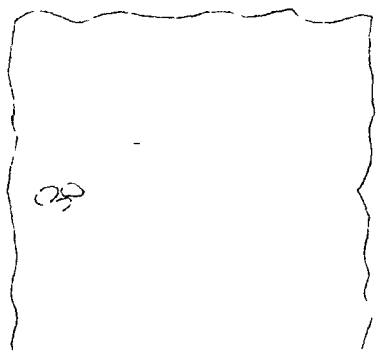

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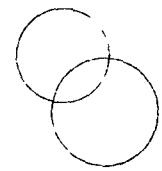

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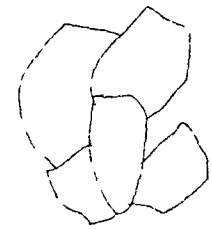

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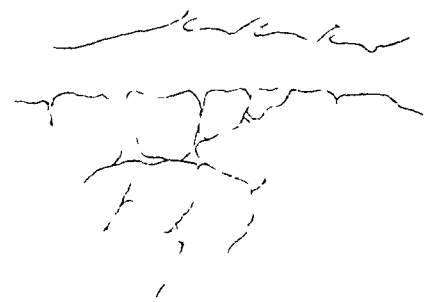

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mucous tissue as above described The cells are larger and the meshwork wider in the part farthest from the vessels The intercellular substance, even at this age, contains connective-tissue fibres, and their number increases in older specimens, hence the necessity for having as young a fœtus as possible to see the uncomplicated characters of this tissue (*Indicate these corpuscles in* Pl V, Fig 6)

If a similar preparation be teased with needles and stained with methyl-aniline before it is mounted, it is easy to isolate flattened endothelial plates with wing-like expansions

ADENOID TISSUE

This tissue forms the basis of the lymphatic glands and the spleen, and occurs widely distributed as simple lymphatic glands or in diffuse patches in many organs of the body It was formerly believed to consist of branched corpuscles, whose processes anastomosed with the processes of adjoining cells, so as to form a network, which was filled with lymph-corpuscles We now know, however, that it is built on the same type as connective tissue It consists of a ground substance, dense reticulum or network—*adenoid reticulum*—made up of excessively fine homogeneous fibrils and membranes, which form a honeycomb-like open network Transparent, flattened *connective tissue* or *endothelial cells* with oval flattened nuclei are applied here and there to these fibres, and partially embrace or enclose them just as in connective tissue The large nuclei which usually occur at the nodes of the network, and which were formerly regarded as the nuclei of the branched cells, are really the nuclei of the flattened cells applied to the fibres This meshwork is filled with *lymph-corpuscles* These are small cells exactly like colourless blood-corpuscles, with a small round nucleus, which is thus easily distinguished from the large oval nucleus of the endothelial plate They are not all, however, of the same size At first sight only these lymph-corpuscles are seen, and so densely packed are they that one requires to shake or pencil them out of the meshwork before the adenoid reticulum can be brought into view

PREPARATION (*a*)—Place a small piece of a fresh lymphatic gland of an ox, or an entire gland from the abdominal cavity of a cat, dog, or rabbit, for a week in Muller's fluid Make transverse sections Stain a section deeply with logwood, and then place it in a test-tube with water and shake it briskly for five minutes or so This detaches the lymph-corpuscles, and the previous staining enables one to see the section Float it on to a slide, and mount in Farrant's solution

(*b*) **Nitrate of Silver Injection and Logwood**—Into a fresh mesenteric or lymphatic gland, thrust the nozzle of a hypodermic syringe filled with a quarter per cent silver nitrate solution, and push in the silver solution into the gland so as to form an artificial œdema The gland must be tinged with the silver solution Place it for twenty-four hours in alcohol and then make sections by freezing At first the sections will be colourless, but on exposure to light, wherever the silver has penetrated, they will be stained brown Stain a section in logwood, and mount it in Farrant's solution or dammar See also lymphatic gland (p 77)

EXAMINATION (L)—Indistinct indications of the adenoid reticulum here and there crowded with lymph-corpuscles stained blue will be seen Select a spot where few corpuscles are to be seen and examine it with (H) Study the excessively delicate adenoid reticulum, with here and there a large oval nucleus lying on it, especially near a node These are the

nuclei of the endothelial plates Observe the small lymph-corpuscles in the meshwork Not unfrequently in the mesenteric glands of the ox certain of the lymph-cells will be found to contain a brownish-coloured pigment, and I have found multi-nucleated cells closely resembling those found in the spleen The source of the pigment in the one case, and the origin and function of these multi-nucleated cells, have still to be investigated In the silver preparation certain parts acted on by the silver are brown, while those unacted on are bluish from the logwood stain The silver opens up the network somewhat, and thickens the fibrils of which it is composed, so that it is more easily seen It forms an instructive preparation (*Indicate the characters of adenoid tissue in* Pl V, Fig 7)

BONE

PREPARATION Methods of softening bone (*a*) **Chromic and nitric acid**—Dissect out a long bone—the femur or humerus of a cat or rabbit—remove the muscles attached to it, but retain the periosteum With a saw cut the bone into pieces three-quarters of an inch in length, and place them in a bottle with 300 cubic centimètres of one-sixth per cent solution of chromic acid (p xxxi) After three days substitute a quarter per cent solution of chromic acid, and after three days more a half per cent for another three days Then transfer the pieces of bone to a large quantity of a mixture of chromic and nitric acid (p xxviii) The object is to remove the calcareous matter and leave the structure of the bone intact The chromic acid alone 'fixes' the elements and partially decalcifies the bone, but to employ chromic acid solution alone requires a long time to complete the decalcification, hence the use of chromic and nitric acid mixture, which completes the process more rapidly, with no risk of the more delicate structures in the bone being injured by the action of the nitric acid It expedites the process to change the fluid frequently At the end of three weeks the bones still retain their form, though flexible and soft, and can now be cut with a knife By thrusting a needle into the bone one can determine when all the calcareous matter has been removed, there being an absence of grittiness The bone will now have a greenish colour, from the deposition of a chromic sesquioxide Place the bone in a large quantity of water, frequently changed to get rid of the acids Either make sections (transverse and longitudinal) at once in the usual way, or place the bones in alcohol till they are required

(*b*) **Picric acid method**—Place similar pieces of bone in a saturated solution of picric acid and add a few crystals of the acid to keep the solution saturated, as part of the acid is used up to combine with the lime in the bone This process takes much longer time than the above, but it answers admirably for fœtal bones or for very small bones After decalcification wash the bones for a long time in water, to get rid of the intensely yellow colouring matter Place them in spirit till they are required Make transverse sections of the shaft of a bone, including the periosteum, decalcified in either of the above ways Place some of the sections in a one per cent solution of osmic acid for twelve hours Wash them thoroughly and preserve them in weak spirit

(*c*) **A 10 per cent solution of sodic chloride and hydrochloric acid.**—This is for showing the fibrillar structure of the bone-matrix (v Ebner and De B Birch) This method is fully described at p xxxiii The sections must be preserved in a ten per cent solution of sodic chloride

(*d*) **Artificial digestion with trypsin**—See p xxxiv

T S SHAFT OF A DENSE BONE DECALCIFIED

EXAMINATION (L) (*a*) **Osmic acid section**—Float the section on to a slide from weak spirit, remove the surplus spirit, add a drop of melted glycerine jelly (p xlviii), and cover (Glycerine and Farrant's solution render the tissues too transparent.) Observe the periosteum surrounding the ring of bone If the section be made through the centre of the shaft there will be little cancellated tissue—only a ring of dense bone surrounding the medullary cavity In the bone observe the Haversian canals, some divided transversely, others obliquely, and some are opened into longitudinally, arranged with reference to these are the bone-corpuscles lying in their lacunæ, which appear like little dark specks Some lacunæ are arranged with reference to the periphery of the bone The lamellæ of bone may be faintly seen Those arranged round the Haversian canals are the Haversian lamellæ, and these with the lacunæ and their canaliculi and Haversian canal make up a Haversian system, the peripheric lamellæ arranged with reference to the periphery of the bone, and lastly some segments of larger circles which lie jammed up between the Haversian systems, though not belonging to them—the intermediary lamellæ, which are segments of greater circles than the Haversian lamellæ This indicates that at one time they were situated under the periosteum, but in process of development they have come to lie amongst the Haversian systems They never contain a Haversian canal (*Indicate these general characters in* Pl VI, Fig 1)

(H) Observe the outer and inner layers of the periosteum and perhaps a layer of somewhat flattened cells lying directly on the bone between it and the periosteum—the osteoblasts Observe the bone-matrix with the faintly indicated lamellæ, and the bone-corpuscles—irregular, somewhat shrunken, nucleated masses of protoplasm, each lying in a space or *lacuna*, with faint indications of the canaliculi The Haversian canals, opened into longitudinally, may be seen to contain osteoblasts and a blood-vessel (*Indicate these structures in* Pl VI, Fig 2)

(*b*) **Stain a T S of the shaft with picrocarmine** for ten minutes, and mount it in Farrant's solution or glycerine jelly Cover (L) The periosteum is now well defined, its outer layer being stained red, from its consisting chiefly of white fibrous tissue, whilst the deeper layer is somewhat yellower in colour The matrix of the bone is yellow and the bone-corpuscles red

(H) The outer layer of the periosteum is now seen to consist of bundles of fibrous tissue crossing each other, mixed with a few elastic fibres, the deeper yellow-coloured layer consists of a large proportion of elastic fibres, mostly arranged longitudinally, and so their cut ends appear as dots, and lying on the bone is a layer of flattened nucleated cells stained yellow—the osteoblasts

L S OF A DENSE BONE DECALCIFIED

EXAMINATION (L)—Observe the Haversian canals cut into and arranged for the most part longitudinally, with here and there transverse or oblique canals connecting them, the rows of lacunæ—five or more between two Haversian canals, the lacunæ are flattened, with their long axis in the axis of the bone, and from each surface they give off canaliculi If the periosteum has been preserved the osteoblasts may be traced into a Haversian canal opening on the surface of the bone (*Indicate the Haversian canals and lacunæ in* Pl VI, Fig 3)

(H) Observe the lacunæ with their included bone-corpuscles, in the Haversian canals the remains of blood-vessels and osteoblasts

BONE LAMELLÆ

PREPARATION—(*a*) With forceps pull off a thin layer of the peripheric lamellæ from a bone softened in dilute hydrochloric acid (p xxxiii) and denuded of its periosteum Examine in water If it is to be preserved, it must be placed for twenty-four hours in one-half per cent osmic acid, and mounted in Farrant's solution

(*b*) Digestion of a piece of bone with artificial pancreatic juice (p xxxiv), previously softened with dilute chromic acid, enables one to isolate the fibres of which the lamellæ are composed (Birch)

The sections must be preserved in a ten per cent solution of sodic chloride

EXAMINATION (H)—The thin sheet of tissue consists of fibres resembling white fibrous tissue, which appear to cross each other in two directions, and here and there a hole may be seen, through which a Sharpey's fibre had passed Projecting from the surface may be seen finger-like processes, these are Sharpey's fibres, which have been pulled out of their sockets

SHARPEY'S FIBRES

PREPARATION—Decalcify a piece of a human parietal bone in dilute hydrochloric acid Make vertical sections, and place one on a slide With needles tear off the peripheric lamellæ, using a dissecting microscope (p xxxv) Some of the fibres will thus be torn from their sockets, and will project as fine processes from the lamellæ

EXAMINATION (L and H)—Observe the fine elongated nail-like process, and perhaps the socket from which they were removed

In the foregoing preparation of a lamella they are seen directed towards the observer No Sharpey's fibres, such as are described above, are found within the Haversian systems They are found in the peripheric and intermediary lamellæ

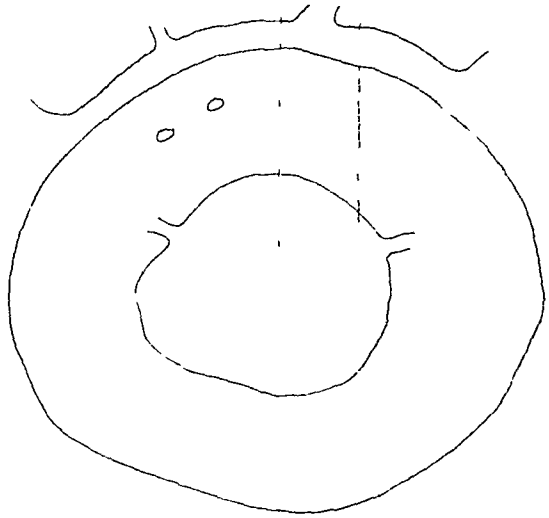
ELASTIC FIBRES IN BONE

In addition to these fibres of white fibrous tissue, elastic fibres also pass into the bone from the periosteum These are best seen in transverse sections of a decalcified shaft of a long bone, which has been slightly stained with glycerine to which a trace of magenta solution has been added (magenta-glycerine) The magenta stains these fibres of a deep red, while the white fibres are unaffected by the dye Further, the elastic fibres branch, which the white never do The magenta-glycerine must be allowed to act slowly, and when the section is sufficiently stained, remove it, and substitute for it Farrant's solution, and cover

FŒTAL BONE

This ought to be softened, preferably with picric acid solution (p xxxii) Select a long bone of a human fœtus or the bones of a newly born kitten They require a shorter time for decalcification than adult bones Make transverse sections and preserve them in preservative fluid (p xl) until required Stain a section with picrocarmine, and mount it in Farrant's solution and cover

PLATE VI BONE



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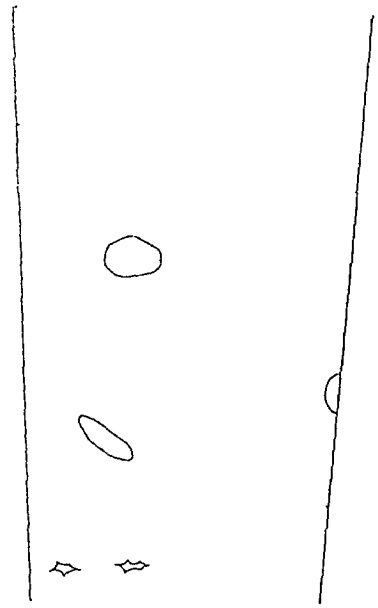

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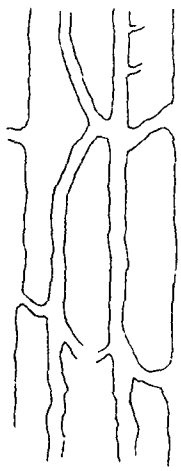

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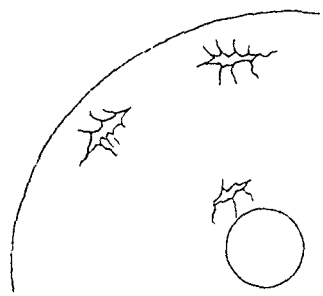

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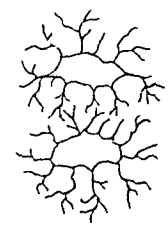

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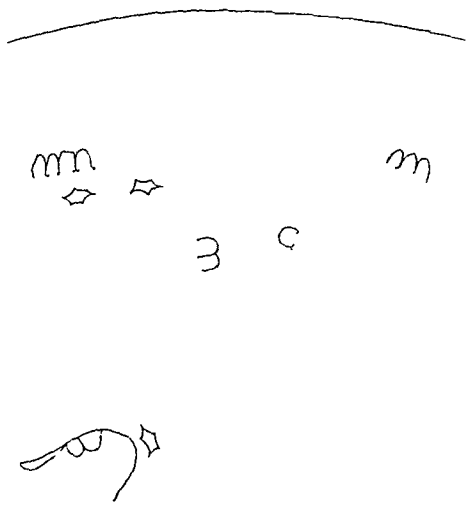

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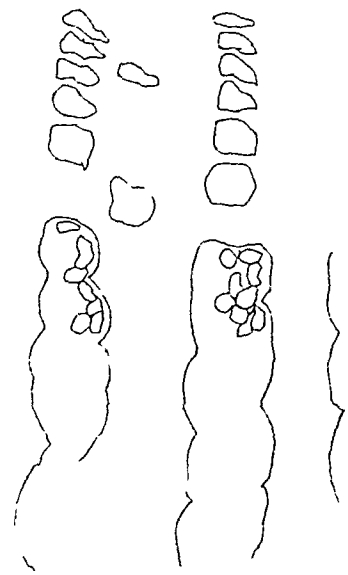

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T S OF DENSE FŒTAL BONE (DECALCIFIED)

EXAMINATION (L) —The bone-matrix is yellowish and the corpuscles in the lacunæ red Observe the ring of bone, not so dense as in the adult, the Haversian canals are relatively larger, and contain a layer of osteoblasts, which line them, and also a blood-vessel The outer layer of the periosteum is red and the deeper layer is yellowish, and between it and the bone a layer of nucleated, somewhat cubical cells—the osteoblasts—which may be traced into Haversian canals opening on the surface of the bone (H) Observe the osteoblasts lying on the bone, and here and there they may be traced into Haversian canals which run horizontally and communicate with the surface of the bone The bone-corpuscles are relatively larger than in adult bone After a few days, they become quite red and are better defined The canaliculi may also be traced Under the periosteum giant-cells may be found here and there (*Indicate the periosteum, the osteoblasts, and bone-corpuscles in* Pl VI, Fig 6)

DENSE DRY BONE

PREPARATION —Take a human radius which has been completely freed from grease, with a fret-saw cut a very thin transverse section Grind the section on a hone moistened with water. Examine with a low power to determine when the section has been ground thin enough Rinse it in water to get rid of the *débris*, and allow it to dry Take Canada balsam, which is quite hard in the cold, and place a small piece of it on a slide, gently warmed over the flame of a spirit-lamp till the balsam melts Allow the balsam to cool, and before it has become quite hard, place on it the section of bone, and cover This method prevents the balsam from getting into the lacunæ and canaliculi, and so rendering the bone too transparent Make a corresponding longitudinal section of the shaft of a long bone

T S OF DENSE DRY BONE

EXAMINATION (L) — Observe the Haversian systems, each consisting of a Haversian canal surrounded by lamellæ disposed concentrically with relation to it—Haversian lamellæ, amongst these are seen the lacunæ which resemble black dots with fine branches—the canaliculi—proceeding from them Observe also the circumferential or peripheric, and the intersystemic or intermediary lamellæ

(H) Observe the shape—flattened ovals—and arrangement of the lacunæ, they appear black, being filled with air From them proceed fine, branching canals, which appear as fine black lines, running across the lamellæ and uniting the lacunæ by a network of very fine tubes The canaliculi of the outermost row of lacunæ of any Haversian system do not usually open into the lacunæ of an adjoining system, but bend on themselves, and open into the lacuna from which they sprang, or into an adjoining lacuna of the same system These are the so-called 'recurrent' canaliculi (Ranvier) (*Indicate the lacunæ and canaliculi in* Pl VI, Figs 4 and 5)

L S OF DENSE DRY BONE

EXAMINATION (L)—The Haversian canals are cut longitudinally or obliquely (with transverse or oblique branches connecting them), and they open on the surface or the bone

and into the medullary cavity The lacunæ are long, narrow ovals, parallel to the canals arranged in several rows between adjoining canals

BLOOD-VESSELS OF BONE

PREPARATION—Place the leg-bones of a rabbit whose blood-vessels have been injected with a gelatine and carmine mass (p li), in a three per cent solution of hydrochloric acid for several days, until they are completely decalcified Place them in water for several hours, and afterwards in a dilute solution of sodic carbonate, to get rid of the last trace of acid Make longitudinal and transverse sections in the ordinary way, and mount them in Farrant's solution

EXAMINATION (L)—Observe each Haversian canal contains a blood-vessel, and note the general arrangement, which follows exactly the Haversian canals (*Indicate the longitudinal arrangement of the blood-vessels in* Pl VI, Fig 3)

CANCELLOUS DECALCIFIED BONE

PREPARATION—Soften the head of a fœtal bone of a kitten or new-born child in the usual way, and make transverse sections through the cancellous head of the bone Stain a section with logwood for five minutes, and another with picrocarmine for fifteen minutes, and mount both in Farrant's solution Cover

EXAMINATION (L)—Observe the open meshwork, the meshes are the cancelli, bounded by trabeculæ of bone, which do not contain Haversian canals In the cancelli are osteoblasts and marrow-cells

(H) Study the osteoblasts, and search for multi-nucleated cells (myoplaques, giant-cells, or osteoclasts)—cells several times larger than the osteoblasts, containing many nuclei imbedded in granular protoplasm They usually lie close on the bone, in a depression which they accurately fill

DEVELOPMENT OF BONE (INTRA-CARTILAGINOUS)

PREPARATION—Place the leg-bones of a rabbit, one week old, or those of a kitten, in picric acid solution, or in the chromic acid and nitric acid fluid, till they are completely softened Make a longitudinal vertical section through the cartilaginous head and shaft of the bone

EXAMINATION (L)—Stain a section with picrocarmine Observe the head of the bone separated from the shaft by the epiphysial cartilage, and below the cartilage the line of ossification In the epiphysial cartilage the cells are arranged to a certain depth above the line of ossification in rows parallel to the long axis of the bone These rows of cells are produced by transverse cleavage of cartilage-cells Below the line of ossification, where true bone commences, are found irregular spaces—the primary medullary spaces—which are bounded by narrow spicules of calcified cartilage, partially covered by a layer of bone In these spaces are seen osteoblasts, young marrow-cells, and blood-vessels

(H) Observe the rows of cartilage-cells, smaller above and larger below At the line of ossification a cartilage-capsule may be found, opening and discharging its contents into a primary medullary space The long spicules have in their centre the remains of the calcified cartilage, and on them is deposited bone, which is now stained of a deep red colour, and osteoblasts may be seen in process of becoming imbedded in this newly formed bone, to form bone-corpuscles In the spaces observe the osteoblasts, and here and there multi-nucleated

cells (osteoclasts), lying on and eroding the newly formed bone of the osseous trabeculæ (*Indicate the rows of cartilage-cells, line of ossification, and other details, in* Pl VI, Fig 7)

L S PHALANX OF A FINGER FROM A HUMAN FŒTUS

After softening, stain with picrocarmine for fifteen minutes, and mount in Farrant's solution

EXAMINATION (L)—Observe the shape of the bone under the periosteum on each side of the shaft is a triangular piece of bone, stained bright red, the base of each triangular portion corresponds to the surface of the shaft, the apices pointing inwards In this piece of bone are seen channels, into which vessels from the periosteum proceed These vessels are accompanied by osteoblasts into the interior of the bone, where already the central part has been excavated to form primary medullary spaces Towards the head of the bone, the cartilage-cells, arranged in rows, are seen just as in an epiphysial cartilage Under the periosteum there are one or more layers of osteoblasts

DEVELOPMENT OF BONE (INTRA-MEMBRANOUS)

One of the three flat bones of the skull of a fœtal kitten or other animal is taken, and after being hardened in picric acid it is stained with picrocarmine and mounted in Farrant's solution

MEDULLARY TISSUE AND MARROW

Ordinary yellow marrow consists chiefly of fat (ninety per cent), but the spongy ends of bones generally and the medullary cavity of the long bones of some animals—*e g* guinea-pig—contain **red marrow**

PREPARATION—Take the leg-bone of a rabbit or guinea-pig, and with an axe or chisel cleave it longitudinally With the point of a knife take out a little of the red marrow, and diffuse it in a drop of salt solution on a slide, and cover

EXAMINATION (H)—Observe the marrow-cells of various shapes—many of them resembling colourless blood-corpuscles, though they contain a large clear nucleus, others are identical with colourless blood-corpuscles Large multi-nucleated cells, many times the size of a coloured blood-corpuscle, may be seen They are the myeloplaques, osteoclasts, or giant-cells, and consist of a granular mass of protoplasm, containing a large number of nuclei in their interior Dilute alcohol reveals these nuclei, and frequently causes a small, transparent, bulla-like mass to appear on the side of the cell (Stirling)

A permanent preparation of red marrow may be made by placing a piece of a long bone of a guinea-pig, opened, as described above, in dilute alcohol for two days Then put a little of the marrow on a slide, and stain it with picrocarmine or logwood, and mount the preparation in glycerine

TAIL OF A RAT DOUBLY-STAINED

PREPARATION **Gold chloride and an aniline dye**—See p xlvii, where this process is fully described

EXAMINATION (L and H)—The tendons are stained with gold chloride, while the bone is stained of one colour and the cartilage of another This is one of the most beautiful preparations in the whole range of histology

MUSCULAR TISSUE

There are two varieties of muscular tissue, the non-striped and the striped The former may easily be obtained from certain of the hollow viscera, and the latter from the voluntary muscles of the limbs

NON-STRIPED MUSCLE (INTESTINE)

PREPARATION —Kill a rabbit, wash out a piece of the small intestine with salt solution, and afterwards distend the gut with absolute alcohol Place the distended gut in absolute alcohol for twelve hours After it is hardened, whilst still distended, with a pair of forceps strip off a thin layer of the longitudinally disposed muscular fibres, forming the outer muscular layer of the gut, and place it in water to remove the alcohol, float it on to a slide and stain with logwood for five minutes and mount it in dammar Cover

EXAMINATION (L) —Observe the large number of fusiform nuclei arranged more or less parallel to each other

(H) Observe the fusiform shape of the nuclei stained deep blue These are the nuclei of the fusiform muscular fibre-cells of which the tissue is composed (*Indicate the nuclei in* Pl VII, Fig 1)

It is necessary to isolate these fusiform cells to show that the tissue is composed of a number of them cemented together

PREPARATION —Place a small piece of the fresh muscular wall of the intestine or stomach in a twenty per cent solution of nitric acid for twenty-four hours, this will dissolve the cement Place a strip of this softened tissue in water to remove the acid, and then tease a small piece on a slide It is very difficult to stain the nuclei of these isolated cells after the action of nitric acid, but a watery solution of magenta may be tried, or steeping in picrocarmine for forty-eight hours or longer Mount in glycerine and cover

EXAMINATION (H) —Notice a great number of elongated small fusiform cells floating in the field Each cell is broad at the middle and tapers towards the extremities Near the centre is the fusiform nucleus stained red, and at each of its poles not unfrequently a few granules are to be seen In some of the fibres corrugated bars may be seen passing across the cell, these are where partial contractions of the body of the cell have been fixed by the acid (*Indicate these fibres in* Pl VII, Fig 2)

BLADDER OF A FROG

PREPARATION —Distend the bladder of a frog with dilute alcohol (p xxxiv), and place it distended in a large quantity of the same fluid for twenty-four hours At the end of that time slit open the bladder, place it on a slide with the inner surface uppermost, and with a camel-hair pencil brush away all the epithelium lining the bladder, which is very easily removed Wash the tissue in water, and stain one piece with logwood and mount it in dammar, and another for half-an-hour with picrocarmine, and mount it in Farrant's solution

EXAMINATION (L)—Observe the large number of nuclei stained either blue or red, lying usually in one plane

(H) Observe the shape and disposition of the long fusiform nuclei Many of the cells are fusiform, but others are tri-radiate

T S OF NON-STRIPED MUSCLE

PREPARATION—Place a piece of the muscular wall of the intestine of a cat in chromic acid and spirit solution, and after two weeks make transverse sections of its circular muscular fibres Stain a section with picrocarmine for twenty-four hours and mount it in Farrant's solution Stain another with logwood for five minutes, and mount it in dammar

EXAMINATION (H)—Observe the field mapped out into small polygonal areas of different sizes These are the fibres cut across These areas occur in groups of small fasciculi, separated from each other by connective tissue, which forms a sheath for them—the *perimysium* In some a nucleus may be found, in others none, because the line of section has not been through the situation of the nucleus in the fibre The variable size of the areas, and the absence of a nucleus in some of them, enable the observer to distinguish easily between the cut ends of non-striped muscular fibres and the mosaic formed by the ends of a number of columnar epithelium cells turned towards the observer, all of which are of the same size, and each one is nucleated Observe the cement-substance between the fibres This is best seen when the illumination is slightly diminished by turning off a little of the light with the mirror Observe also that the fibres are arranged in groups or bundles, surrounded and separated from each other by connective-tissue septa, which are continuous throughout—the *perimysium* This is continuous with the cement or *endomysium*, which lies within the bundles between the muscle-cells (*Indicate the divided fibres in* Pl VII, Fig 3)

CEMENT-SUBSTANCE OF NON-STRIPED MUSCLE

PREPARATION—Kill a rabbit, and wash out a piece of the small intestine with distilled water Fill the intestine with a half per cent solution of nitrate of silver, and place the distended intestine for ten minutes in a quarter per cent solution of nitrate of silver Owing to the difference of concentration of the fluids on the two sides of the gut, diffusion of the silver takes place and so complete saturation of the tissues is obtained Wash away the silver in ordinary water and expose the gut in alcohol to a strong light After it has become brown, with a pair of forceps tear off a thin lamina of the outer longitudinal muscular wall of the gut, steep it in water and float it on to a slide and mount it in Farrant's solution Another strip may be stained with logwood solution and mounted in dammar, if it be desired to reveal the nuclei of the muscle-cells Another method of obtaining the outer layer of the muscular coat is to place a small piece of the gut with its external surface next the glass slide, and then with a knife to scrape away layer after layer from within outwards until only the longitudinal and the serous coats remain

EXAMINATION (H)—Observe a large number of fine black lines disposed for the most part longitudinally When carefully traced they are seen to bound narrow fusiform spaces or areas These silver lines indicate the existence of a cement-substance which holds the fibres together Lying between the fibres are elongated channels with bulgings upon them, and lined by sinuous epithelium These are the silvered lymphatics, *i e* lacteals We shall use this preparation for the study of the general characters of lymphatics (*Indicate the silver lines in* Pl VII, Fig 4.)

INTRA-NUCLEAR PLEXUS OF FIBRILS IN NON-STRIPED MUSCLE

The student who desires to show this arrangement must proceed as follows —

PREPARATION—Kill a newt and place the small intestine and mesentery in a five per cent solution of ammonium chromate for twenty-four hours Wash in water till no colour is given off, then transfer it to picrocarmine for several hours With scissors snip off a small piece of the mesentery and mount it in glycerine Cover

EXAMINATION (H)—Observe the very large spindle-cells, either single or disposed in groups Study a nucleus and observe in its interior a delicate plexus of fibrils In its interstices lies a clear homogeneous substance On carefully focussing with one-eighth of an inch objective, fibrils may be traced through the poles of the nucleus, and be found to be continuous with fibrils disposed in the long axis of the cell These muscular fibres are examined on account of their large size (Klein) (*Indicate these plexuses of fibrils in* Pl VII, Fig 5)

MESENTERY OF NEWT DOUBLY-STAINED WITH PICROCARMINE AND LOGWOOD

PREPARATION—Prepare the mesentery of a newt with ammonic chromate as directed above After washing away all the chromate from a piece of the mesentery, stain it for fifteen minutes in dilute picrocarmine (ten drops to a watch-glass of water) Wash it in water and place it in water acidulated, with a few drops of acetic or picric acid for an hour After washing place it in dilute logwood solution, until it assumes a faint lilac colour, taking care that it is not over-stained Mount it in glycerine

STRIPED MUSCLE

This may be studied in any of the voluntary muscles A muscle consists of striped muscular fibres held together by connective tissue and supplied with blood-vessels and nerves The fibres must be examined in the fresh condition, and after the action of reagents

FRESH STRIPED MUSCLE

PREPARATION—Kill a frog, and from one of its muscles—preferably the sartorius, because it is composed of parallel fibres—snip off a small piece and tease it slightly in salt solution Cover

EXAMINATION (H)—Observe the cylindrical fibres consisting of the sarcous substance marked with alternate light and dim stripes (Pl VII, Fig 8) In some places the striation may be obscure or the stripes very close, while here and there slight longitudinal fibrillation is observable Irrigate with *distilled water* and after a short time a fine bleb may be seen on the side of one or more of the fibres This is the *sarcolemma* (*Indicate the sarcolemma in* Pl VII, Fig 6) Water has passed through the sarcolemma and raised it from the sarcous substance Irrigate the preparation with *dilute acetic acid*, when the nuclei, lying under the sarcolemma and in the sarcous substance, are revealed (Pl VII, Fig 7) They are elongated spindles, sometimes slightly coloured, lying in the long axis of the fibres The reason why the nuclei are not seen at first is because they have the same refractive index as

the surrounding sarcous substance Another way of showing the sarcolemma is to take a piece of fresh muscle, and after teasing it, to press a needle across a fibre so as to try and break the fibre If this be accomplished, the sarcous substance will be ruptured, and contracting leaves the structureless transparent sarcolemma stretched between the ends of the ruptured fibre

CLEAVAGE OF THE SARCOUS SUBSTANCE

PREPARATION —Place small, narrow strips of any muscle of a cat or rabbit, which has been dead for a few hours, in a small quantity of a sixth per cent solution of chromic acid for a week The muscular fibres will tend to split up *longitudinally* into *fibrils* Chromic acid seems to dissolve a cement

(2) Place similar pieces of muscle in hydrochloric acid—one part to fifty of water for a week After this time each fibre tends to split transversely into *discs* Artificial digestion (p 92) in an acid medium produces the same result in a few hours

EXAMINATION (H) —Take a small piece of the muscle which has been steeped in chromic acid, and after washing it thoroughly tease it out with needles in glycerine Each muscular fibre tends to split longitudinally into excessively fine threads—the *fibrillæ* or *fibrils*—each one being marked with light and dim bands like the original fibre (*Indicate the fibrillæ in* Pl VII, Fig 10)

Take a similar piece of the hydrochloric acid muscle and tease it The fibres split across into thin narrow discs or 'muscle-discs' Occasionally a muscular fibre splits up both longitudinally and transversely at the same time, when Bowman's sarcous elements are formed These are sometimes seen at the end of a fibre broken across by chance (*Indicate the discs in* Pl VII, Fig 9)

MUSCLES OF ARTHROPODA

To trace the finer details in the structure of muscle it is customary to use the striped muscles of insects, such as the common water-beetle—Dytiscus marginalis or Hydrophyllus piceus—or the common cockroach I find, however, that the muscles of the common edible crab answer the purpose admirably, and are far better suited for use in a large class

PREPARATION —(*a*) Place a beetle or the amputated limb of a crab in absolute alcohol, and if possible keep some of the muscles on the stretch when so doing Leave them there for a week (*b*) A mixture of one part of methylated spirit and three parts of Muller's fluid, for three to four weeks, hardens muscle most admirably The tissue must be kept in the dark, else reduction of the chromic salts takes place very rapidly Scoop out a small piece of the muscle of a crab and steep it in water Steep one piece in picrocarmine for twenty-four hours, and stain another with logwood for a few minutes Tease them thoroughly with needles Mount the former in glycerine and the latter in dammar Both preparations show much the same structure, only what is red in the one is logwood-tinted in the other

EXAMINATION of the picrocarmine preparation (H) —Notice the large size of the muscular fibre, and how easily it splits into fibrillæ Each fibre shows well-marked transverse striation Study this The broad, dim discs (contractile discs) are stained red, and the narrower, less refractive discs (interstitial discs) are slightly yellow In each dim disc a series of fine vertical rods may be seen Study carefully the light disc—across its centre runs a fine line, Dobie's line or Krause's membrane—which divides the disc into two *lateral* discs This line is continuous with the sarcolemma, and is supposed to represent a membrane by which

the muscle-fibre is divided into a series of compartments, each containing a dim disc with a lateral disc attached to each end In this preparation no nuclei are seen (*Indicate these discs in* Pl VII, Fig 11)

Logwood and Dammar Preparation (H)—The dim discs are logwood-coloured—the light discs unaffected In the course of the fibre are seen oval nuclei under the sarcolemma Otherwise the details are the same as in the above preparation

If the muscle of an insect—say hydrophyllus or dytiscus—be taken and similarly treated, a row of fine granules is observable in the lateral discs These form the granular layer of Flogel

LIVING MUSCULAR FIBRE

PREPARATION—This is not particularly well adapted for a large class of students Cut off the head of a water-beetle and then open its chitinous body with a sharp-pointed pair of scissors, so as to expose the large wing-muscles Snip off a small piece of these and tease it rapidly in aqueous humour (p xxx) or salt solution (p xxx) Cover and examine

EXAMINATION (H)—Observe the fibres, their striation, and that they are soft and plastic Here and there a thickening, progressing along a fibre—a contraction-wave—with approximation of the discs, may be observed

NUCLEI OF MUSCLE

PREPARATION—Take a small piece of a muscle of a rabbit or cat which has been preserved in alcohol, tease it out in water, and stain it in carmine till it is of a bright red colour Wash it in water to remove the surplus carmine Place on it a large drop of glacial acetic acid, and after doing so examine it with a low power, to notice when the nuclei alone remain stained The effect of the acid is to remove the diffuse staining, and leave the carmine only in the nuclei Mount it in either Farrant's solution or dammar—preferably the latter

EXAMINATION (H)—Observe the striation, and in the course of the fibres oval nuclei with their long axis in the axis of the fibres, stained deeply red (*Indicate the nuclei in* Pl VII, Fig 8)

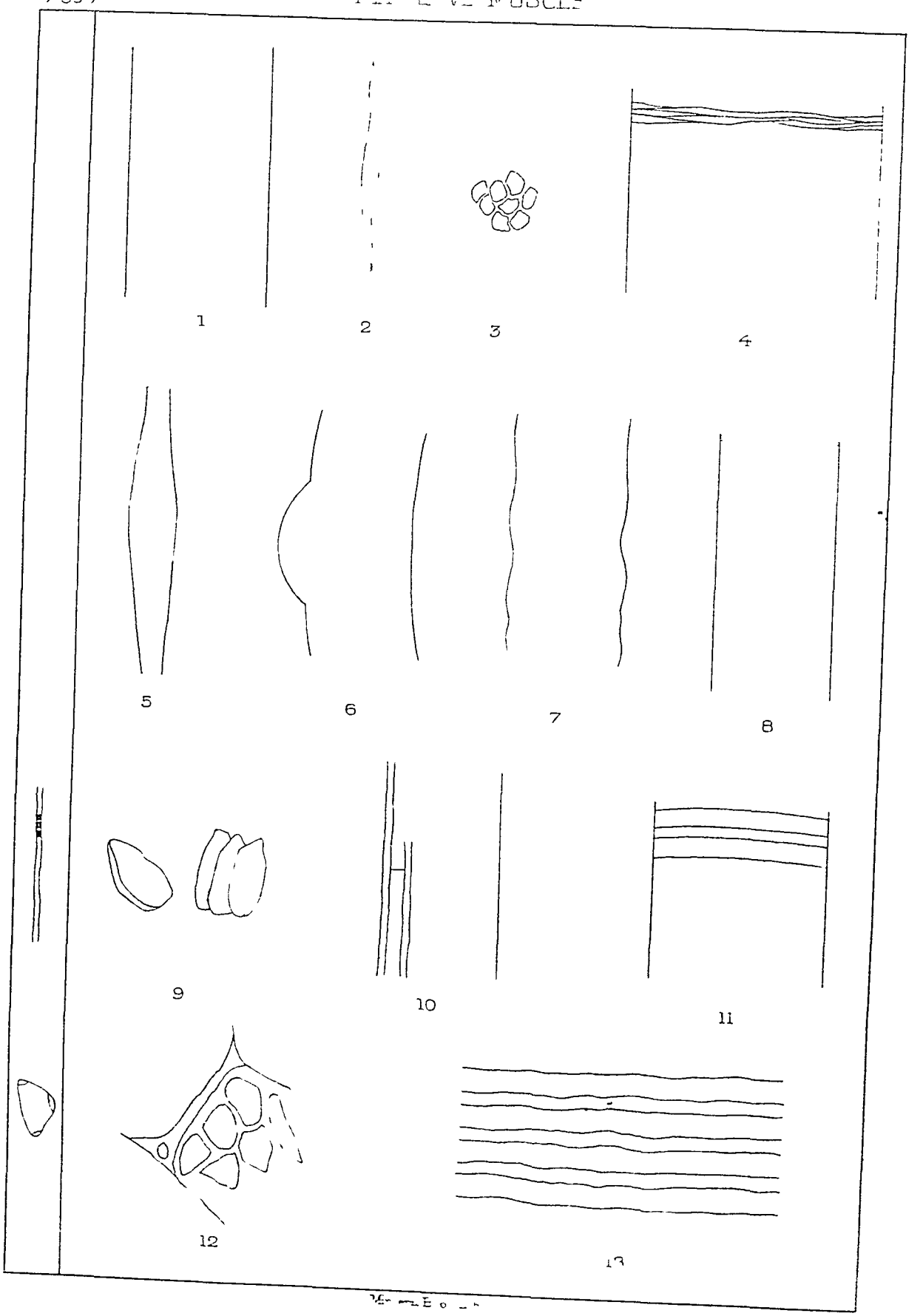
Relation of the Nuclei to the Sarcolemma—Take a limb-muscle—or the tongue—of a cat or rabbit or other animal, previously hardened in alcohol, or chromic acid and alcohol (p xxxi), and make a transverse section by freezing or other means Stain it for a few minutes with logwood and mount it in dammar This preparation will also show the relation of the *connective tissue*, or *perimysium*, to the muscular fibres

EXAMINATION (L)—Notice the connective-tissue—perimysium—of a light blue colour surrounding groups of muscular fibres cut transversely, which appear as small polygonal areas stained light blue (*Indicate the connective tissue, and the cut muscular fibres with their nuclei in* Pl VII, Fig 12)

(H)—Observe the cut ends of the fibres, each more or less polygonal, through mutual pressure of the fibres against each other Under the sarcolemma are seen two or more small logwood-stained nuclei in each fibre, and the cut end of the sarcous substance appears slightly dotted The nuclei are often situated at the angles of the polygon Trace the connective tissue in very fine strands between the fibres—forming the *endomysium*—and, it may be, containing a capillary here and there In a transverse section of a frog's muscle nuclei are found to exist, not only under the sarcolemma, but also throughout the sarcous substance

×300

PLATE V. MUSCLE



BLOOD-VESSELS OF MUSCLE

PREPARATION—Make longitudinal and transverse sections of any muscle of a cat or other animal whose blood-vessels have been injected with a carmine or Prussian blue and gelatine mass (p li) The sections may be very slightly stained in logwood, or mounted unstained in dammar, or a small piece of the muscle may be simply teased and mounted in Farrant's solution

EXAMINATION of a longitudinal section (L) Observe the capillaries arranged parallel to the long axis of the fibres—one capillary between each two fibres, with here and there transverse branches connecting them They lie outside the muscular fibres and amongst the delicate connective tissue which supports them (*Indicate the arrangement of the capillaries in* Pl VII, Fig 13)

Transverse section (L)—Observe the cut ends of the fibres and between and outside the sarcolemma the cut ends of the capillaries The larger blood-vessels lie in the connective tissue of the perimysium

It is advisable to inject the blood-vessels of the lower limbs of a rabbit with a Prussian blue and gelatine mass Place the limbs in a one per cent solution of bichromate of potash After the mass has set, select one of the red muscles, *e g* the semi-tendinosus Most of the muscles of the rabbit are pale or almost colourless The above-mentioned muscles, and some others (soleus, adductor minus, muscles of the jaw), are, however, distinctly red, the semi-tendinosus, at least, has a peculiar distribution of its blood-vessels Tease out a small piece of one of the injected red muscles and mount it in glycerine or dammar

EXAMINATION (L)—The blood-vessels have the same distribution and relations as described above, but they present this peculiarity—that on the transverse, short capillaries are small dilatations like little aneurysmal swellings These swellings do not exist in the blood-vessels of the pale muscles of the same animal Further, the capillaries usually pursue a more tortuous course, and the veins, also, sometimes present similar fusiform swellings in their course

The muscular fibres of the heart will be examined in connection with that organ (p 47), and the nerves of muscles with the terminations of nerve-fibres (p 45)

Fœtal developing muscle may be studied in any embryo after hardening in Muller's fluid It is characterised by the great number of its nuclei, the smallness of the fibres, and the imperfect striation In fresh fœtal muscle iodine solution reveals the presence of glycogen

RELATION OF MUSCLE TO TENDON

PREPARATION (*a*) **Centrum tendineum of the diaphragm**—Cut out the diaphragm from a newly killed rabbit, place it in lemon juice for five minutes, and, after steeping in one per cent gold chloride for an hour, transfer to a twenty-five per cent solution of formic acid for twenty-four hours During this time the preparation must be kept in the dark till the gold is reduced Snip out a small piece of the muscle, with the central tendon attached, and tease it in glycerine on a slide and cover, or a section may be made parallel with the long axis of the muscular fibres

EXAMINATION (L and H)—Observe the transversely striped muscle, and trace it towards the tendon, where it ends abruptly in a conical form which is lodged in a corresponding depression in the tendon

NERVE-FIBRES

There are two varieties of nerve-fibres —

1 The medullated or white
2 The non-medullated or gray

MEDULLATED NERVE-FIBRES

Each nerve-fibre consists of a thin transparent structureless sheath, the primitive sheath, with here and there a nucleus lying under it Within this is the medullary sheath or white substance of Schwann with a double contour forming the greater part of the nerve It is interrupted at intervals by regular constrictions, or Ranvier's nodes, where the white substance is entirely absent In the centre of the fibre is the axis-cylinder, a fine uniform cylinder continuous throughout the whole length of the fibre The proper view to take of the axis-cylinder is that it is directly continuous at its central end with a process of a nerve-cell, and is in fact one of the processes of a nerve-cell enormously elongated and pushed outwards towards the periphery of the body, and covered in whole or part of its course with one or more sheaths This is the only view that explains the remarkable results of degeneration in a nerve-fibre when it is separated from its so-called trophic nerve-centre (Waller) and also explains the so-called union of nerve-fibres, which is in fact no union at all, but is due to a pushing out or growth of the axial cylinder into the old sheaths of the degenerated nerve-fibres from which the axis-cylinder and most of the white substance of Schwann, but not the nuclei, have disappeared

FRESH NERVE-FIBRES

PREPARATION —Kill a frog and remove its sciatic nerve With a pair of sharp scissors cut off half an inch, and place it on a slide in a drop of salt solution With a pair of needles gently tease out one end of the nerve, and examine it from time to time with a low power, to ascertain when it has been sufficiently teased Cover

EXAMINATION (H)—Observe the nerve-fibres of various sizes, some of them about the breadth of a coloured blood-corpuscle, others larger or smaller Note the double contour of the white substance of Schwann which is highly refractive This double contour exists in the living nerve, and is not due to the action of reagents This can easily be proved by examining a medullated nerve in the lung or tongue of a living frog or the lung of a newt Select the end of a fibre which has been broken across, and study the myeline exuding from its cut end The myeline, or white substance, collects into drops, with concentric markings These must not be mistaken for cells, from which they are easily distinguished by the absence of a nucleus (*Indicate the myeline exuding from a nerve-fibre in* Pl VIII, Fig 1)

In the centre of each fibre is a clear uniform band, the axis-cylinder Trace the course of a fibre carefully, and a Ranvier's node may be easily distinguished The white substance is interrupted here, further, if the nerve-fibre be slightly on the stretch, the oblique *incisures* of Schmidt and Lantermann, passing through the myeline, can also be made out Irrigate the preparation with picrocarmine, and continue the examination after twenty minutes Observe that a nucleus under the primitive sheath is stained red, the carmine passes in at the nodes of Ranvier and also stains the axis-cylinder red If an axis-cylinder happen to extend beyond

the myeline, it is quickly stained red by the carmine (*Indicate a node and the incisures in* Pl VIII, Fig 2)

It is remarkable that nearly all the phenomena which have been laboriously made out by the use of special reagents can be seen in a perfectly fresh or even in a living nerve-fibre

EFFECT OF OSMIC ACID

PREPARATION—Kill a frog, dissect out a sciatic nerve Tie a fine thread round each end of the nerve, carefully preserving it from getting dry, and by means of the thread stretch the nerve on a small piece of wood—an ordinary wooden match with a slit at either end to hold the thread answers admirably Place the nerve and wood in a one per cent osmic acid solution for ten minutes The effect of the osmic acid is to blacken the phosphorised fats of the myeline and to 'fix' its elements The nerve is stretched in order that the nodes and incisures may be made quite distinct Remove it and wash it carefully in water, and then place it, still on the stretch, in a test-tube with picrocarmine In twenty-four hours the staining is complete I find it to be very advantageous to leave it in picrocarmine for a fortnight or longer The effect of this is to soften the connective-tissue of the nerve, so that on teasing it at the end of this time the single nerve-fibres are easily obtained Tease a small piece—always tearing off a fibre in its long axis—in a drop of glycerine Cover

EXAMINATION (H)—Observe the myeline, blackened and still retaining its double contour Trace a fibre and observe, at pretty regular intervals, the constrictions or nodes of Ranvier, and between each two nodes a brightly stained nucleus imbedded in a trace of protoplasm, will be found lying under the primitive sheath and partly indenting the myeline (*Indicate two nodes in* Pl VIII, Fig 5) Study a node Observe the absence of the myeline, and that only the slightly red-coloured axis-cylinder passes from one segment of the nerve to another The axis-cylinder, therefore, is the only essential part of a nerve Some excessively delicate nerve-fibres will be seen The delicate connective tissue supporting the nerve-fibres is easily made out (*Indicate a node, the nucleus, and the incisures in* Pl VIII, Figs 3 and 4)

Amongst the medullated fibres may be detected the grey or sympathetic nerve-fibres They are distinguished by being delicate fibres, with a large oval nucleus here and there in their course, and are devoid of myeline, and therefore do not possess a double contour

ACTION OF NITRATE OF SILVER RANVIER'S CROSSES

PREPARATION—(*a*) Tease out roughly a small piece of a perfectly fresh sciatic nerve of a frog in a quarter per cent solution of silver nitrate on a slide, and leave it in this solution for five minutes or so Wash it with water and then tease it carefully in glycerine, cover and expose it to the action of light until it becomes brown

(*b*) The small intercostal nerves of a rat or rabbit may be excised and plunged entire into silver nitrate solution and mounted entire in glycerine, and exposed to the action of light as above

EXAMINATION (L and H)—Observe a fibre and notice the dark brown crosses seen here and there (Pl VIII, Fig 8) The long limb of the cross is produced by the silver penetrating to the axis-cylinder, and staining it, or an albuminous material covering it, for a short distance, whilst the transverse, more deeply stained, bar is due to the cement which unites the so-called segments of a nerve one to another (Pl VIII, Fig 9) Sometimes the longitudinal limb of the cross may be marked transversely by lines (Frommann's lines) If a whole nerve be used, a complete investment of endothelial cells will be found outside all

ARRANGEMENT OF NERVE FIBRES TO FORM A NERVE

PREPARATION—Place pieces of the human or other sciatic nerve, cut into pieces one inch long, into chromic acid and spirit (p xxxi), or Muller's fluid and spirit (p xxxii), for ten days, and after hardening in alcohol make transverse sections in the ordinary way Stain a section in logwood, and, if it be desired, another in carmine, and mount them in dammar They both show the same details

EXAMINATION (L)—The various bundles composed of nerve-fibres are held together by a common framework of connective tissue—the epineurium—arranged so as to form a sheath around and between the various bundles, and in it may be found transverse sections of the large blood-vessels which supply the nerve with blood Round each bundle observe a special sheath—the *perineurium*—composed of lamellated connective tissue with flattened connective-tissue cells between them The spaces between the lamellæ represent lymph-spaces (*Indicate these bundles in* Pl VIII, Fig 10)

Study a nerve-bundle—Observe it is made up of many nerves cut transversely, only the stained axis-cylinders are indicated

(H) Study the epi- and perineurium, observe the cut ends of the nerve-fibres varying in diameter, and the section of the axis-cylinder stained red or violet and surrounded by a transparent circular area, which represents the myeline or white sheath of Schwann, outside this a faint circle, indicating the primitive sheath Between the nerve-fibres may be seen very delicate connective tissue—the *endoneurium*—with here and there a small blood-vessel in it (*Indicate the stained axial cylinders and the cut ends of the nerve-fibres in* Pl VIII, Fig 11) If the hardening process has been continued for a month or longer, concentric rings may be observed in the white substance of Schwann (*Indicate these in* Pl VIII, Fig 12) Longitudinal sections of a nerve prepared as above may also be studied

THE CONNECTIVE-TISSUE ELEMENTS OF A NERVE

PREPARATION—Take a small fragment of a nerve which has been hardened in one-sixth per cent solution of chromic acid for ten days, and after staining in logwood, tease it thoroughly in a drop of glycerine

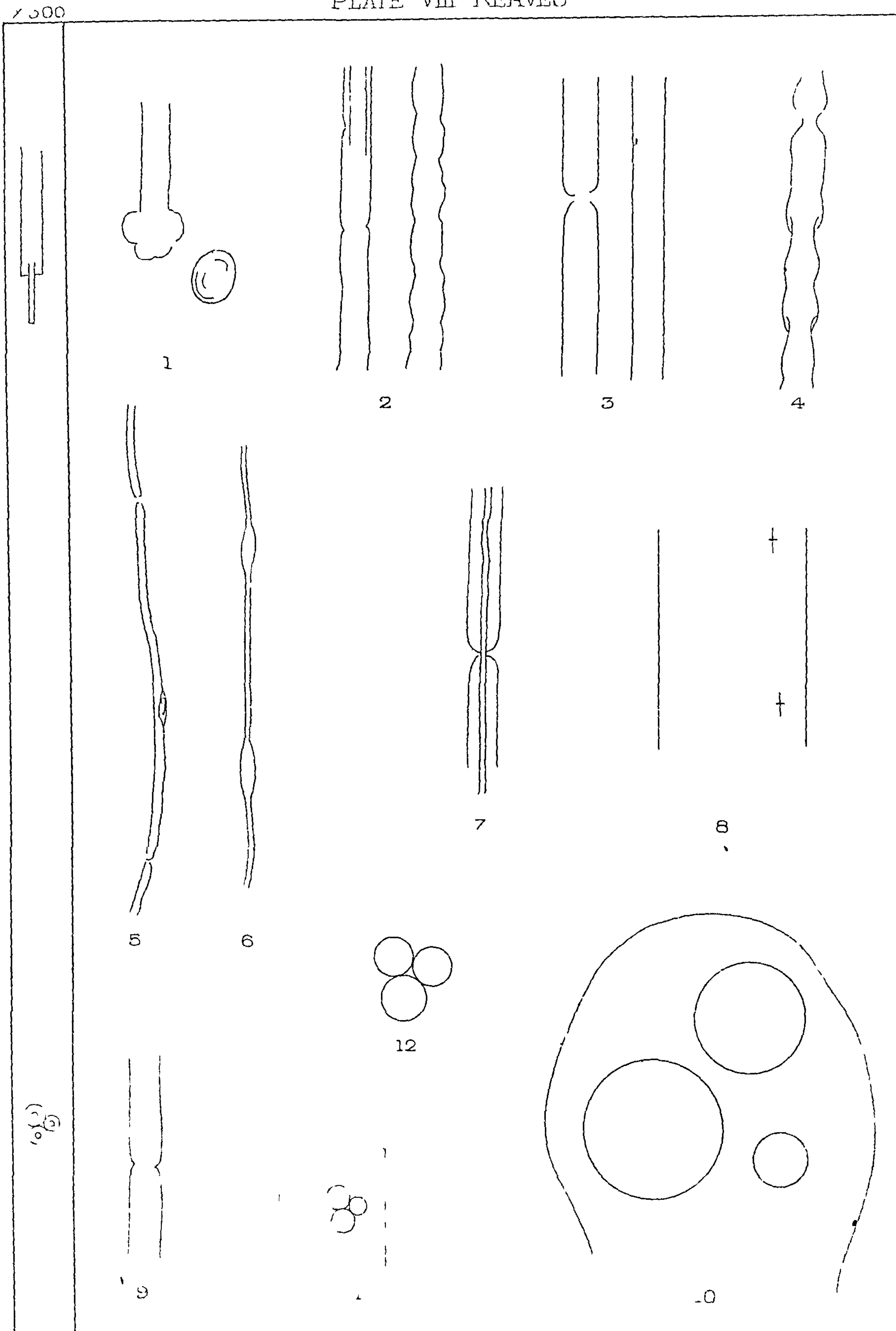
EXAMINATION (H)—Observe the nerve-fibres The axis-cylinder, stained violet, will easily be recognised amidst the coagulated myeline It is specially interesting to trace the axis-cylinder across a node of Ranvier Observe the connective tissue, some of it in the form of delicate lamellæ, with here and there oval nuclei stained violet lying on them, and also detached flattened plates—the connective-tissue corpuscles—each containing a large oval violet nucleus

FRESH NERVE-FIBRES OF THE SPINAL CORD

PREPARATION—With scissors snip off a small fragment of the white matter of a fresh spinal cord of an ox or sheep, place it on a dry slide, do not add any fluid, put on a cover-glass, and compress it into a thin layer

EXAMINATION (H)—Observe the drops of myeline, easily recognised by their concentric markings and the absence of a nucleus, and very delicate nerve-fibres with bulgings or ampullæ upon them, due to the pressure of the cover-glass on the nerve-fibres, which here are

PLATE VIII NERVES



Eastern Bros. Lith

devoid of a primitive sheath There are no ampullæ in the living condition Do not preserve this preparation

NON-MEDULLATED NERVE-FIBRES

These may be detected in an ordinary nerve and studied there, but it is better to select a nerve containing these or sympathetic fibres in large quantities The sympathetic nerve from the neck of a rabbit may be selected, but it is far better to have a larger nerve This is easily obtained from the spleen of an ox Accompanying the branches of the splenic vein are large nerve-trunks, the nerves of the spleen, which contain only a few medullated fibres, the rest being non-medullated nerve-fibres With scissors cut open the splenic vein, and then the nerve-trunk is easily dissected out

PREPARATION —Place the nerve, extended, as for the medullated nerve, in a one per cent. solution of osmic acid, and after washing place it in picrocarmine for twenty-four hours Tease a small fragment in glycerine, and cover

EXAMINATION (H) —Neglect the medullated nerve-fibres and observe the non-medullated nerve-fibres They are pale, flat, slightly granular bands which have no myeline, and in their course the oval red-stained nuclei of the primitive sheath are easily seen Sometimes the nuclei are seen on edge, and at other times on the flat In this nerve many 'plasma cells' (p 20) are found

If desired, a fresh nerve may be teased in salt solution, when similar appearances are observable

Nerve-cells will be considered with the organs in which they occur

SOME PERIPHERAL TERMINATIONS OF NERVES

We shall only allude here in detail to the terminations of nerves in striped muscle by means of end-plates, and to the Pacinian corpuscles attached to the ends of certain sensory nerves (p 46) Other modes of termination will be described in the organs where they occur

TERMINATIONS IN STRIPED MUSCLE

PREPARATION —This is by no means an easy task In mammals the best muscles are the straight muscles of the eyeball of a rabbit—the muscular part of the diaphragm and any thin muscles such as the intercostals In the lizard (*Lacerta agilis*) the end plates may be more easily found But the best muscles are those which attach the skin to the ribs of the smaller snakes In any case it is best to employ the following gold method Snip out very thin pieces of the muscle to be examined, and place them in lemon-juice for five minutes, then wash in water and transfer to a one per cent solution of gold chloride for twenty minutes or half an hour—wash again and place the preparation in the dark for twenty-four hours in a twenty-five per cent solution of formic acid After washing thoroughly, to remove the acid, tease out a small piece of the muscle in glycerine, and search with a low power for a nerve-fibre, or trace the outline of a muscular fibre till an end plate is obtained The axis-cylinder is continued beyond the end plate, and breaks up into fibrils inside the sarcolemma, their ultimate relation to the sarcous substance, however, is unknown These preparations are difficult to make, and do not keep well afterwards

The terminations of nerves in unstriped muscle will be considered under the head of small intestine (p 71), the same methods are applicable as for those of striped muscle nerve-endings

PACINI'S CORPUSCLES

PREPARATION—These are numerous in the mesentery and in the mesorectum of the cat, where they are easily obtained free from fat The student may also find them in the deeper layers of a vertical section of the skin of the hand or foot, or in the pad of a cat's foot, but I have found them most easily for students in transverse sections of an entire fœtal leg or arm Being placed relatively closer to each other in the fœtus, they are, like sweat-glands, more easily observed there than in adult textures

In the cat's mesentery they can be seen as small, oval, transparent, hard bodies Snip out one of these and examine it at once in salt solution with a low power, the nerve entering it may be seen, as also the central core surrounded by concentric laminæ If a permanent preparation be desired, excise a piece of the mesorectum or mesentery containing these bodies, pin it out in a stretched condition on a flat piece of cork, and place the cork, tissue downwards, for a week, in a two per cent solution of potassic bichromate, after that wash away all trace of the reagent with water, snip out a corpuscle and stain it slowly (twenty-four to forty-eight hours) in dilute logwood, which must be changed several times Wash in water and mount in glycerine As the corpuscles are of considerable thickness, it may be necessary to place a thin layer of paper under the edges of the cover-glass to avert pressure, and allow the cover-glass to lie flat upon the preparation

EXAMINATION (L)—Observe the nerve-fibre like a stalk approaching and entering the central core of the oval corpuscle, which latter is made up of laminæ one outside the other (H) Trace the nerve-fibre, and observe that the medullary sheath stops where the nerve enters the corpuscle Only the axial cylinder passes into the central core Study the concentric laminæ, which exhibit a series of nuclei on each surface These are the nuclei of the endothelial cells which cover each lamina

To study the laminæ and their endothelial covering, a corpuscle, hardened as above, must be broken up with needles, by which process the various laminæ are readily separated from each other

Transverse sections will be obtained in one or other of the preparations already mentioned These show the concentric distribution of the laminæ round the central core, and also the nuclei of the cells between each two laminæ (p 92)

HEART AND BLOOD-VESSELS

THE heart is invested by a fibrous pericardium covered by a layer of squames, whose existence is easily demonstrated by the nitrate of silver process It is lined by a thinner membrane also covered with squames, the endocardium, capable of demonstration in a similar manner The muscular tissue of the heart is transversely striped, though involuntary The striæ are always more or less indistinct The fibres branch and anastomose They consist of short nucleated segments joined by a cement, and are devoid of a sarcolemma

ISOLATED MUSCULAR FIBRES OF THE FROG'S HEART

PREPARATION—Place the heart of a newly killed frog in a small quantity of a forty per cent solution of caustic potash (p xxxiv) for a quarter of an hour Take a fragment of the tissue and tease it with needles in a drop of potash solution Be careful to add no water Cover

EXAMINATION (H)—Observe the isolated muscle-cells They are fusiform, and in the broad middle part there is a well-defined nucleus, and the substance of the fibre is transversely striped Do not preserve this If it be desired to possess a permanent preparation, small pieces of a frog's heart must be placed in dilute alcohol for twenty-four or forty hours, and then stained in picrocarmine , and, after teasing to isolate individual cells, add glycerine and mount the preparation

FRESH HEART OF A MAMMAL

Treat a small piece of the heart of a rabbit in the same way as the frog's heart Cover

EXAMINATION (H)—Observe the oblong muscle-cells isolated Each cell is nucleated and transversely striated Do not preserve this

HEART FOR PRESERVATION

PREPARATION—Place small pieces of the heart of a rabbit, or a piece of a human heart, including a piece of the pericardium, in chromic acid and spirit fluid for ten days, transfer to spirit, and then make a transverse section through a ventricle, including the pericardium Stain a section with logwood and mount it in dammar

EXAMINATION (L)—Observe the fibrous pericardium stained of a light blue, and note its thickness Here and there it sends fine processes into the heart, to form a perimysium for the bundles of muscle It is important to observe the normal thickness of the fibrous covering, and also the normal amount of connective tissue present, as in every organ, to be enabled to judge when there is any alteration in the thickness of the capsule or the amount of the interstitial connective tissue This point is specially important in relation to the pathological histology of organs Not unfrequently in these trabeculæ are to be seen sections of the

coronary artery and vein and cardiac nerves, easily recognised by their lying together and by their structure Trace the muscular fibres mapped out into bundles Some are cut transversely, others obliquely, and some longitudinally Many nuclei are seen, which are the nuclei of the muscular fibres Select a transverse section of a group of fibres (H) Observe the polygonal shape of the fibres from mutual pressure, the existence of a violet-stained nucleus in the centre of some of them—not in all, and the striæ radiating from the nucleus, a delicate endomysium will also be seen (*Indicate these in* Pl IX, Fig 2) Select a longitudinal section, observe the faint striation, the branching and anastomosing of the fibres, and the oval, violet-stained nuclei in the course of the fibres The places where the oblong muscular cells which make up a fibre join, indicated by a transverse line, due to the presence of a cement-substance, are to be looked for (*Indicate the characters of these fibres in* Pl IX, Fig 1)

Cement-substance —The cement-substance which unites one cell to another may be demonstrated thus Thrust the nozzle of a hypodermic syringe filled with half per cent solution of silver nitrate amongst the fibres of the heart, and forcibly inject the silver solution so as to separate the heart-fibres Place the heart in alcohol, and after twenty-four hours snip out a small piece which has been acted on by the silver, and after teasing in glycerine expose it to the light and examine for the lines of junction of the cells stained brown or black by the silver

Blood-vessels of the heart present practically the same arrangement as in striped muscle, and are prepared and examined in the same way (p 41)

Nerves and ganglia of the heart —Sometimes preparations of these are obtained in transverse sections of the ventricles, but to see their arrangement and distribution the heart of a frog, or small pieces of any mammalian heart, must be treated with lemon-juice, chloride of gold, and formic acid, as described at p xlv The nerves and ganglia must then be dissected out with the aid of a dissecting microscope (p xxxv)

PURKINJE'S FIBRES

A delicate network of fine transparent lines is seen, with the naked eye, on the interior of the ventricles of the heart of a sheep or ox These are Purkinje's fibres They consist of imperfectly developed muscular fibres—they are, in fact, vestiges of what each heart-fibre was originally They are made up of polygonal-shaped cells, each often with two nuclei, and only the outer half of each cell is striated, but in the deeper layers, cells are found where the striation has involved three-fourths of a cell, and deeper down still they become continuous with the ordinary heart-muscle Each muscular fibre of the heart, and in fact, every striped muscle, was originally non-striped, and the striation always begins at the periphery of the cell, so that the fibres made up of these cells represent an embryonal phase of muscular tissue

PREPARATION —With scissors or a knife cut out these fine threads and macerate them for two days in dilute alcohol, stain with picrocarmine, and tease a small piece in glycerine until isolated cells or groups of cells are found

EXAMINATION (H)—Observe the isolated polygonal cells, each with two nuclei, and the outer half of each cell marked with every gradation between these and cells where the perinuclear part is completely striated (*Indicate these cells in* Pl IX, Fig 3)

BLOOD-VESSELS

There are three well-marked varieties of blood-vessels—*arteries, veins, and capillaries* A typical artery has three coats—outer, middle, and inner In a middle-sized artery the inner coat consists of a layer of elongated squames with their long axis corresponding to the long axis of the vessel, outside this, delicate connective tissue and an elastic membrane or lamina, either with holes in it—'fenestrated membrane'—or it consists of a sheet of elastic tissue which in sections is thrown into folds, owing to the contraction of the muscular coat outside it It is to be remembered, however, that these folds do not exist in a living vessel distended with blood The middle layer consists of circularly-disposed non-striped muscular fibres, with a small amount of elastic and white fibres The outer coat (adventitia) consists of fibrous tissue with longitudinally disposed elastic fibres, which are specially numerous next the muscular layer In the largest arteries (aorta) there is relatively a large amount of elastic tissue in the inner and middle coats, and in the middle coat the non-striped muscle and elastic plates occur in alternate layers In the veins the division into coats is not so sharply marked The middle coat contains less muscular fibre-cells and more connective tissue, and the outer coat not unfrequently contains non-striped muscle, especially in large veins The capillaries consist of a layer of nucleated, flattened, epithelial cells, joined at their edges with a cement-substance

Small arteries and capillaries are best studied in the pia mater

SMALL ARTERIES AND CAPILLARIES

PREPARATION—Get the head of a recently killed sheep, and remove the brain With a scalpel remove as much of the brain as possible, leaving the pia mater With a stream of salt solution wash away the remainder of the brain-substance It is most important to remove all the brain-substance, else it forms a granular deposit which obscures the preparation Place the pia mater in chromic acid and spirit solution for a week After washing it thoroughly in water, snip out a small piece and stain it with logwood, and mount it in dammar A similar preparation may be stained with picrocarmine, and mounted in Farrant's solution

EXAMINATION (L)—Select a small artery, and study its mode of branching and how these branches terminate in capillaries Note in the artery the transversely disposed nuclei of the muscular fibres of its middle coat (Pl IX, Figs 6 and 7) Observe the outer coat Search for a vein with its much thinner coat and fewer transversely disposed nuclei—*i e* muscular fibres—and compare it with an artery

(H) **Select a small artery** Observe its outer and middle coats, and, composing the latter, non-striped muscular fibres, disposed in one or more layers The fusiform nuclei of the muscular coat are very apparent The folds of the elastic lamina of the inner coat may be seen **Study a capillary** It appears as a perfectly structureless membrane, with oval nuclei imbedded in it, which bulge into its lumen It requires the use of nitrate of silver to demonstrate its endothelial characters

DEVELOPMENT OF CAPILLARIES

The development of capillaries is easily studied in the tail of a half-grown tadpole prepared by the ordinary gold chloride method To see the capillaries distinctly it is well to remove the superficial epithelium after staining If the lemon-juice and gold method (p xlv) be employed, the formic acid employed afterwards removes the superficial epithelium The omentum of a new-born rabbit presents easily recognised whitish patches, or 'milk spots' (Ranvier) in it, which are aggregations of corpuscles like lymph corpuscles, and others, which become continuous with buds given off from existing blood-vessels These buds ultimately become channelled to form blood-vessels

FRESH BLOOD-VESSELS

Take the pia mater, washed free from cerebral matter, and tease a small piece on a slide in salt solution Cover An examination reveals the same general arrangement as described above, but the nuclei of the muscular coat of the arteries requires the addition of acetic acid to bring them into view Add acetic acid, to observe this It also swells up the adventitia, makes it clear and transparent, and the elastic lamina when present, with its folds, is brought clearly into view—and so are the nuclei in the capillary wall Do not preserve this

AORTA

PREPARATION—Take small pieces of the human aorta or of the aorta of an ox, and place it in picric acid for twenty-four hours Make longitudinal and transverse sections in the usual way, and stain them with picrocarmine, taking care not to wash the sections too much in water, and mount them in Farrant's solution

EXAMINATION (L)—Observe the fusion of the different coats and the large amount of elastic fibres and plates in the different layers All elastic tissue is stained yellow, connective tissue pink, and non-striped muscle light reddish-brown, so that these different tissues are easily distinguished (H) Note the numerous layers of elastic fibres in the inner coat, in a transverse section they appear as bright yellow granules, in the longitudinal as yellow lines Note the large amount of elastic tissue in the middle coat, where it occurs in the form of elastic laminæ

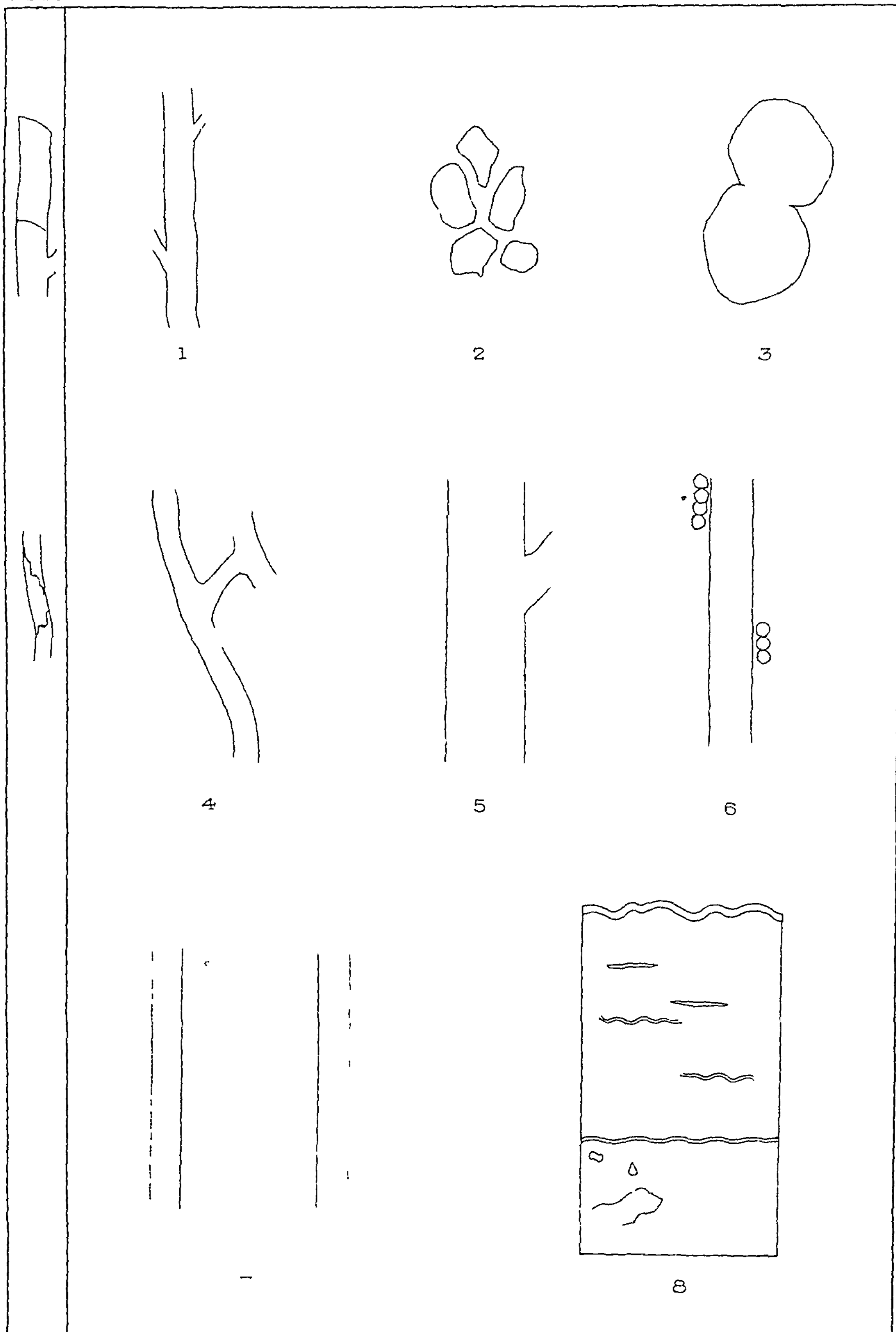
If it be desired to see the lining epithelium, silver (p xlv) the inner surface of the aorta of an animal just killed, and mount a thin shaving in dammar

ISOLATED ELASTIC LAMINÆ

PREPARATION—By far the best method is the one I described several years ago for the isolation of elastic tissue, viz, to digest thin strips of the aorta in artificial gastric juice (p 92) till everything except the elastic element is dissolved (De B Birch) Wash the tissue, to get rid of acid, and mount in Farrant's solution Examination reveals all gradations, from a network of elastic fibres to fenestrated membranes and sheets of elastic tissue The elastic fibres not unfrequently present transverse markings

x 300

PLATE IX HEART & BLOOD VESSELS



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MIDDLE-SIZED ARTERY

PREPARATION—Place a moderate-sized artery—say the femoral or posterior tibial of a child, or the basilar artery of a sheep or man—in chromic acid and spirit for a week, and make transverse sections Stain one section with logwood, and mount it in dammar, cover, another with picrocarmine, and mount in Farrant's solution

EXAMINATION (L)—Observe the inner coat, perhaps with the layer of squames lining it Observe the elastic lamina, as a clear, yellow, bright line thrown into folds by the contraction of the middle coat which surrounds it If a large artery be under examination, a sub-epithelial layer of delicate connective tissue may be found between the epithelium and the elastic lamina The elastic lamina is an important guide in disease of the blood-vessels, for if changes take place internal to it, it is a disease of the inner coat, if immediately external to it, the disease is in the middle coat The student should, therefore, familiarise himself with its appearance, on account of its important pathological relations The middle coat consists chiefly of several layers of circularly disposed non-striped muscular fibres The nuclei are long ovals and of a violet colour Between the fibres may be found elastic tissue Outside this note the adventitia, consisting mainly of white fibrous tissue stained blue, and containing elastic fibres, especially in its inner part (*Indicate the epithelium, elastic lamina, and other coats in* Pl IX, Fig 8)

(H) Study the elastic lamina, it is a good landmark in a section On its inner surface a profile view may be obtained of the lining epithelium Observe the middle and outer coats

The **Epithelium and its Cement-substance in blood-vessels** is demonstrated by the use of nitrate of silver For a class this is easily managed, thus —

PREPARATION—Kill an etherised rabbit by bleeding Open the thorax and place a cannula in the aorta, and (after making an opening in the inferior cava), wash out the blood-vessels with distilled water Then inject the vessels with a half per cent solution of silver nitrate Cut out the intestines and wash out their contents, and then expose them and their mesentery in a mixture of equal parts of spirit and water to sunlight till they become brown Select either a piece of the mesentery which contains blood-vessels, or what answers equally well, take a piece of the small or large intestine and lay it on a slide with its mucous surface upwards With a scalpel scrape away the mucous coat and mount the muscular and serous coats in dammar

EXAMINATION (L and H)—Select a small artery, notice the silver lines running transversely, indicating the disposition of the circular muscular fibres, and inside these black silver lines mapping out narrow lanceolate areas with their long axis in the axis of the vessel which are the squames lining the artery Try and find a vein and compare the epithelium of the two vessels Trace an artery until it splits up into capillaries, and study the silver lines in a capillary These indicate that it is made up of elongated flattened endothelial cells If it be desired to reveal the nuclei in these plates, a section should be stained with logwood (*Indicate the silver lines in a capillary in* Pl IX, Fig 4, *and in the artery in* Fig 5)

VEINS

Exactly the same methods are employed for bringing into view their structure Many sections of them will be found in other organs They are recognised by their thin walls, the absence of so perfectly defined an elastic lamina, their thinner muscular coat, and the less distinct demarcation of their individual coats

CIRCULATION OF THE BLOOD

The circulation of the blood may be studied in any vascular transparent organ, *e.g.* web of frog's foot, lung of frog, and also in the mesentery. For the student it is best to study the circulation in the web of a frog's foot.

METHOD.—Select a frog with a slightly pigmented web, and with a hypodermic syringe inject into the lymph-sac under the skin of the back four minims of a quarter per cent. watery solution of curara, and place the frog under a bell jar till its motor nerves are paralysed, *i.e.* till it ceases to move when it is pinched. Place the frog on a piece of card-board one and a-half inches wide and six long, with a triangular slit of one-inch base cut at one end of the card. Tie a thread round the tips of two adjoining toes, *e.g.* the third and fourth. Fix the threads in a slit made in each horn of the cardboard, and stretch the web gently across the slit. Moisten the web, place the card-board on the stage of the microscope, and fix it with clips.

EXAMINATION (L).—Observe the arteries, with the blood moving in them from the large to the smaller vessels. In the centre of the stream a more rapid current, and between it and the wall of the vessel the slower stream or lymph-space. Select a vein, known by its thinner walls and the direction of the blood-stream, and notice the movement from the smaller to the larger vessels, and the stream slower than in the arteries. Next observe the network of capillaries, with the blood-corpuscles moving in single file. Notice also the pigment-cells, some of them branched, but if they are contracted they look like small black specks. Cover the web with a fragment of a cover-glass. (H) Study a capillary, *observe its thin wall and the passage of* the corpuscles in single file through it. The coloured corpuscles are elastic, as shown by the way in which they twist and easily become distorted, and as easily regain their normal shape. Select a small vein and observe the colourless corpuscles dragging lazily along in the lymph-space and adhering to the wall of the vessel.

If desired, the phenomena of inflammation of the web can easily be studied by applying a small quantity of mustard moistened with water, or some other irritant, for a few minutes. Wash off the irritant from the web and study the effects.

RESPIRATORY ORGANS—LARYNX, TRACHEA, AND LUNGS

THE *epiglottis* has already been examined (p 18) The preparation may be referred to again Its anterior surface is covered by stratified epithelium, and so is the posterior, but here the submucous coat contains adenoid tissue, and a large number of mucous glands (p 54), which not unfrequently are lodged in depressions of the yellow elastic or reticular cartilage, which constitutes the basis of the organ Not unfrequently taste-bulbs are met with amongst the epithelium on the posterior surface

The *arytenoid cartilages* consist of yellow fibro-cartilage, and sections of them show transition stages from ordinary hyaline to yellow fibro-cartilage (p 19) The submucous tissue contains many mucous glands

If desired the *vocal cords* may be hardened in chromic acid and spirit mixture, and vertical sections made in the ordinary way

TRACHEA AND LUNGS

PREPARATION—Kill an etherised cat by bleeding, open the thorax, and allow the lungs to collapse Tie a cannula in the trachea, and with a syringe distend the trachea and lungs with a mixture of chromic acid and spirit (p xxxi) Suspend the lungs in a large quantity of the same fluid Change the fluid at the end of twenty-four hours, after three days cut the trachea and lungs into small pieces, and place them in fresh hardening fluid for a week or ten days, and then, after washing them thoroughly, transfer them to alcohol until sections are required Preserve a piece of human trachea in the same way Distend a piece of human lung and preserve it as above Make transverse and longitudinal sections of the trachea, transverse sections through a bronchus as it enters the lung, and sections through the pleura and subjacent lung, and also across a bronchus within the lung-substance Preserve the sections in preservative fluid (p xl) until they are required

Sections of such an organ as the lung, with its numerous open spaces, ought always to be made after steeping in gum, which fills the spaces, and after freezing the organ is, as it were, solid, and the gum can easily be dissolved out by water Nothing is so good as a freezing microtome for making sections of lung

The lungs are covered externally by a serous membrane, the *pleura* Its epithelial covering can easily be demonstrated by the silver process (p xlv) on the lungs of a cat just killed After silvering, a superficial slice is taken and mounted in dammar or glycerine If the lung be silvered while it is distended *ad maximum*, *i e* in a state of inspiration, the cells appear as polygonal squames, if it be collapsed, *i e* in a state of expiration, the cells appear slightly cubical, showing that the cells are soft and accommodate themselves during life to the changes of size in the air-vesicles during the respiratory acts

TRANSVERSE SECTION OF TRACHEA OF A CAT

Stain it for half an hour in picrocarmine and mount in Farrant's solution

EXAMINATION (L)—Observe the arc of hyaline cartilage forming nearly two-thirds of a circle and deficient posteriorly The cartilage is invested by a fibrous perichondrium stained red It is continuous with the layer of circularly disposed non-striped muscular fibres, that connect the ends of the cartilages and help to make up the posterior wall of the trachea This muscle, the trachealis muscle, is not inserted into the tips of the cartilage-rings, but into the outer surface of the fibrous perichondrium, some distance from their free ends The muscle is easily made out, for it has a dull red appearance Outside the cartilage is some fat and connective tissue Observe the mucous membrane with its columnar ciliated epithelium, and under it *the submucous coat, containing many mucous glands, most of the acini* of which lie internal to the cartilages, and it may be some adenoid tissue The sacs of each mucous gland are enclosed in a connective-tissue capsule, and have a flattened appearance where they lie inside the cartilage, but are round or oval between the rings Between the different groups of glands single fat-cells, or small groups of fat-cells, may be seen (*Indicate the general characters in* Pl X, Fig 1)

(H) Observe the epithelium in several layers, the upper layer cylindrical and ciliated It rests on a basement-membrane not easily seen in the trachea of the cat, though easily seen in the human trachea, where it plays an important *rôle* in pathological processes Amongst the epithelium are goblet-cells which are stained of a deep red Under the epithelium observe beautiful layers of elastic fibres arranged longitudinally, which appear as rows of bright yellow dots lying amongst the connective tissue Observe the mucous glands, and their acini inside the cartilages, and trace a duct to its opening on the surface in a funnel-shaped manner *The acini of the glands lie in the submucous coat, which in addition to connective tissue contains* a very considerable quantity of adenoid tissue As the gland-ducts pass obliquely to reach the surface, it is difficult to get a complete section of a gland with its excretory duct The ciliated epithelium is continued a short distance into the excretory duct, but it soon gives place to moderately tall cylindrical epithelium, which lines the duct The alveoli or sacs of the gland vary in appearance according to whether they were or were not secreting at death If secretion had been taking place, the cells lining them will be filled with a clear transparent mucus-like mass, and few granular cells are to be seen If there has been no secretion, the gland-sacs will be lined by a regular cubical epithelium of a more or less granular appearance Observe the numerous leucocytes in the submucous coat Observe the non-striped muscle —trachealis—*only present posteriorly and its attachment to the perichondrium* some distance in front of the free ends of the tracheal rings Outside it may be seen transverse sections of nerves and nerve-ganglia As the mucous membrane is very vascular, many sections of blood-vessels will be seen in it The cartilage is ordinary hyaline cartilage, but its corpuscles present a characteristic arrangement Under the perichondrium the cells are flattened, and lie parallel to the surface of the cartilage, whilst those in the interior are elongated or oval and are placed vertically from one surface to the other, *i e* across the long axis of the cartilage (*Indicate the epithelium, glands, and part of the cartilage in* Pl X, Fig 2)

Double-stain a section of a trachea with picrocarmine and iodine green (p xlv) The mucous glands, adenoid tissue, and cartilage are stained green

Vertical section of Trachea, stained with picrocarmine and mounted in Farrant's solution

EXAMINATION (H)—Observe the elliptical pieces of cartilage—the sections of the cartilaginous rings cut vertically Observe the same general arrangement as described in the

transverse section, with this difference, that the mucous glands are seen to be most numerous between the cartilages, and the longitudinally disposed elastic fibres under the epithelium can now be detected Study these various parts under a high power

HUMAN TRACHEA

Transverse section of a Human Trachea, stained with picrocarmine and mounted in Farrant's solution

EXAMINATION (L)—Observe the same general arrangement of cartilage, glands, tracheal muscle, and mucous coat as described in the cat's trachea Notice, however, that the ciliated epithelium is usually absent at some parts, from the difficulty of getting perfectly fresh tissue to preserve An important point is the existence of a well-marked layer of tissue stained of a deep red—a 'basement membrane' so called It lies immediately under the epithelium inside the longitudinally disposed layer of elastic tissue It is a marked feature in the human trachea, and plays an important part in pathological changes, especially in those resulting from chronic bronchitis Observe the mucous glands and their ducts, the acini of many of them lie outside the trachealis muscles, so that their ducts have to perforate it to reach the surface of the trachea The largest glands lie posteriorly, and they present the same characters as previously described, their appearance varying with their state of physiological activity

(H) Where the superficial epithelium has been detached, good examples of developing epithelial cells may be studied Study each of the other structures in detail

The so-called 'basement membrane,' it seems to me, resembles very closely a similar structure which exists in the mucous coat of the cat's stomach, though there its relation to the gland-structures is different I am unable to discover any corresponding structure in the trachea of the rabbit, cat, or dog Its structure, relations, and homologies require further investigation It is certainly quite different from the basement-membrane made up of epithelial cells, and described by Débove as occurring under the epithelium of an intestinal villus, the bladder, trachea, &c

Double-stain a section of human trachea with picrocarmine and iodine green

TRANSVERSE SECTION OF A BRONCHUS AT THE ROOT OF THE LUNG

Place a section in a quarter per cent solution of osmic acid for twenty-four hours, and mount it in Farrant's solution, and another ought to be stained with picrocarmine and similarly mounted

EXAMINATION (L)—Observe the bronchus, and note on each side of it a large blood-vessel, the one a branch of the pulmonary artery, and the other, on the opposite side, a branch of the pulmonary vein These three structures lie in channels, and are imbedded in connective tissue, and are thus accurately mapped off from the adjacent vesicular lung-tissue

Observe the **bronchus** Note that several pieces of hyaline cartilage are inserted in the bronchial wall Outside them is connective tissue often containing a few fat-cells—which are blackened in the osmic acid preparation Besides this, note transverse sections of small blood-vessels—the bronchial arteries, and, lying near them, transverse sections of nerves, with perhaps a ganglion in their course Observe the mucous coat thrown into folds, and under it the cut ends of longitudinally disposed elastic fibres Outside this a continuous ring of non-striped muscular fibre—the bronchial muscle It is perforated here and there by the ducts of

the mucous glands, whose acini lie between it and the cartilages, though they are most numerous where no cartilage exists Perhaps a lymphatic cord may be found in the submucous coat Observe the pulmonary blood vessels and compare the relative thickness of their coats (*Indicate the general arrangement in* Pl X, Fig 3)

(H) The epithelium and glands have exactly the same structure as in the trachea The cut ends of the elastic fibres, stained bright yellow, are most numerous where the mucous membrane is raised as a fold Study the fibrous connective tissue mixed with elastic fibres which exists outside and around the cartilages and blood-vessels Select a transverse section of a nerve, if possible with a ganglion in it The ganglia and large branches of nerves will be found *outside* the cartilages, and usually accompanied by one or more small blood-vessels (bronchial) Observe the ganglionic cells, large oval corpuscles with a distinct nucleus and nucleolus, and between or around them the cut ends of nerve-fibres medullated and non-medullated, the whole surrounded by a fibrous sheath (*Indicate a small part of the wall of a bronchus in* Pl XI, Fig 1)

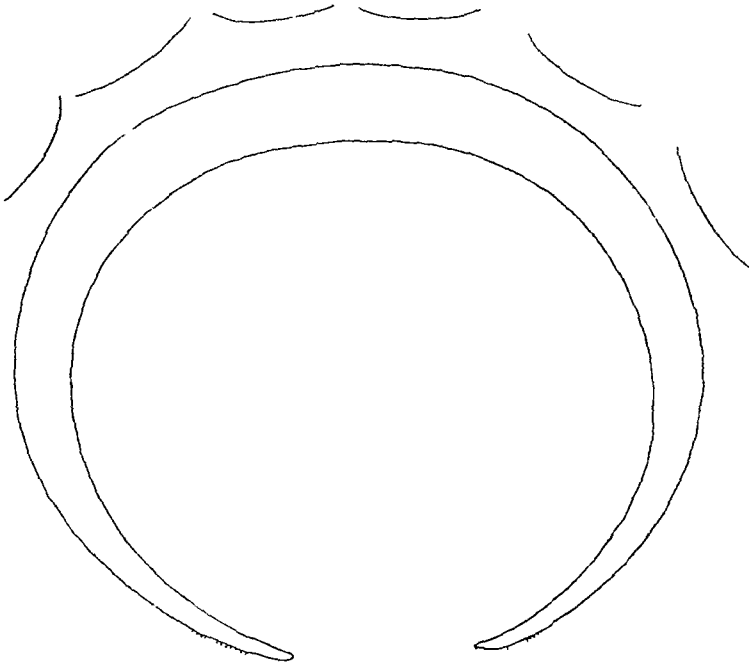
Vertical Section through the Pleura and the Subjacent Vesicular Tissue —Stain with logwood and mount in dammar

EXAMINATION (L) —Observe the pleura, made up of fibrous tissue stained blue, and note its normal thickness From its under surface it sends into the lung, at regular intervals, fine processes of connective tissue—the *interlobular septa*—*i e* processes between adjoining lobules It may be possible to trace the connection of these septa with the connective tissue around a bronchus They contain many lymphatics, and are often pigmented from the presence of charcoal or soot, especially in the human lung They are of great importance pathologically, and this connection of the pleural connective tissue with that which enters the lung at its root is also important, *e g* in the production of dilatation of the bronchi and other pathological processes Here, as in other organs it is important to bear in mind the connection of the connective tissue of the capsule with that in the interior of the organ Observe now the *air-vesicles*, cut in every direction, but notice that those placed most superficially, *i e* next the pleura, are somewhat pyramidal or conical in shape, and have their bases directed towards the pleura, the air-vesicles elsewhere are polygonal in shape, and may or may not have a base to them, which depends on the line of section They are separated from each other by a very small quantity of connective tissue, and by elastic fibres, which form a network round each air-vesicle Here and there a transverse section of a small bronchus may be found It is recognised by the cubical epithelium lining it, and by the presence of cartilage or glands in its walls In the vesicular structure search for a transverse section of an infundibulum The nuclei seen in the air-vesicles belong to the squames lining them, and those forming the capillaries in their walls Search for a small bronchus opened into longitudinally, and observe its dichotomous division and its expansion into air-vesicles (*Indicate the pleura, and the shape of the air-vesicles, in* Pl XI, Fig 2)

(H) Observe the *pleura*, which consists of two layers, the superficial one contains a considerable number of elastic fibres, and the lower, or sub-pleura, consists of looser connective tissue, which is continued into the lung between the lobules as interlobular septa In it may be seen sections of the pleural blood-vessels and lymphatics, the latter appearing as fine slits The endothelium on the surface of the pleura is not visible

Observe an *air-vesicle* Note its outline—rounded or polyhedral If its thin edge be seen in profile, observe the squamous epithelium lining it, though it is better seen where a portion of the wall of an alveolus is flat in the field In the alveolar wall trace the branching elastic

PLATE X TRACHEA & LUNG



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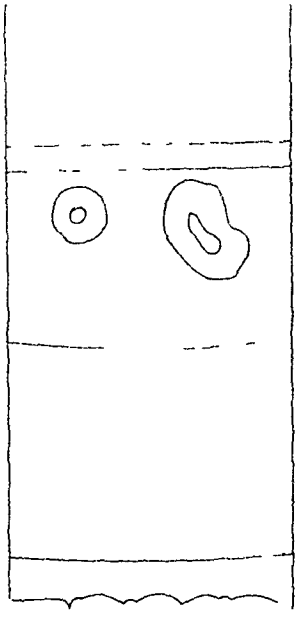

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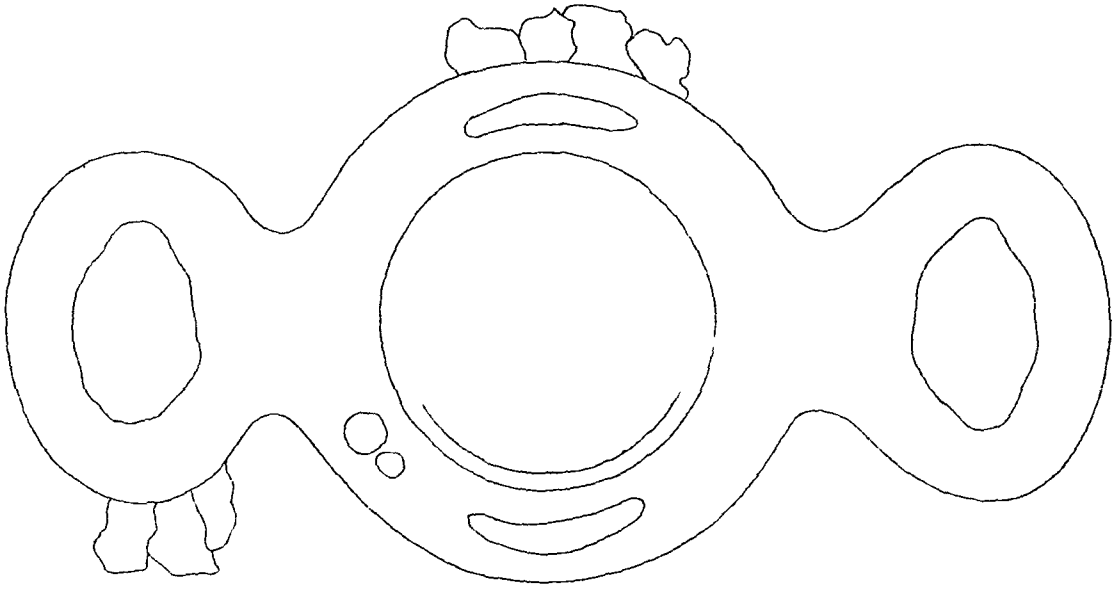

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Mintern Bros lith

fibres, and carefully distinguish the plexus of capillaries, sometimes containing a few blood-corpuscles (*Indicate the epithelium and elastic fibres in* Pl XI, Fig 3) In the capillary wall observe the nuclei, which are not to be confounded with those of the squames lining the air-vesicle In the cat's lung, fusiform nuclei, indicating the existence of non-striped muscle between the vesicles, are to be detected The amount of non-striped muscle between the air-vesicles is sometimes greatly increased in amount in certain diseases It is easy to see this in the lungs of a cat suffering from the presence of the ova of a worm (*Ollulanus tricuspis*) in the lungs (Stirling) Select a very *small bronchus cut longitudinally* Both cartilage and glands have disappeared from its walls, and the epithelium lining it is not so tall as in larger bronchi Trace it into an alveolar duct, which passes into the infundibulum, into which the terminal alveoli open on all sides In the alveolar duct and infundibulum the epithelium consists of low polyhedral cells without cilia The walls of the alveolar ducts and infundibula contain many non-striped muscular fibres, disposed circularly, which are in direct continuity with the bronchial muscle

Picrocarmine Preparation—Stain a similar section with picrocarmine, and mount it in Farrant's solution After several days it shows the above details, even better than the dammar preparation

Osmic Acid Preparation—Place a section of lung, hardened, as above, in chromic acid and spirit mixture, in a quarter per cent solution of osmic acid for twenty-four hours, and, after washing, mount it in Farrant's solution It shows the above details most beautifully, the elastic fibres stand out very prominently, and their arrangement is easily made out

Vertical Section of Human Pleura and Lung—Prepared as above, and similarly mounted Note the more conspicuous interlobular septa, often pigmented from the deposition of charcoal particles in the lymphatics Compare other parts of the section, and note especially the branching character of the elastic tissue, for the physician is sometimes called on to recognise these fibres in the sputum of a person suffering from gangrene of the lung

HUMAN FŒTAL LUNG

Prepare the lung of a fœtus—preferably one that has not respired—in the same way, with chromic acid and spirit mixture, as directed for adult lung In ten days it will be ready for cutting Or, use picric acid solution as the injecting and hardening medium, which will harden it in two days Make sections across a lung, and, after staining with picrocarmine, mount in Farrant's solution

EXAMINATION (L)—Observe the distinct lung-lobules, each covered on its free broad end by a relatively thick pleura, and separated from its neighbouring lobule by thick interlobular septa, whose continuity with the adventitia or connective tissue of the intra-pulmonary bronchus can easily be made out In each interlobular septum note the large open spaces, which are sections of the interlobular lymphatics Study the small non-distended air-vesicles, and note (H) that they are lined by low cubical cells, not yet converted into squames by the distension of the lung as the result of the act of inspiration (*Indicate the pleura and interlobular septa in* Pl XI, Fig 6)

SQUAMOUS EPITHELIUM OF THE AIR-VESICLES

PREPARATION—Remove the lungs of a cat or kitten from the thorax, and distend them with a quarter per cent solution of nitrate of silver, and keep them distended by tying a string round the trachea Place them, with a weight attached, in alcohol until required When

required, cut the lung into pieces, and make sections by freezing Expose the sections to the action of light, until they become brownish in colour Stain a section with picrocarmine for twenty-four hours, and mount it in glycerine, and another with logwood for a few minutes, and mount it in dammar

EXAMINATION (L)—Observe the same general arrangement of the air-vesicles as already described Select an air-vesicle where a portion of its wall is seen on the flat, and examine

(H) Observe the silver lines, indicating the existence of epithelium (squamous), lining the vesicle These lines bound polygonal areas Between these larger and clear areas notice small brown granular areas or polyhedral cells, in groups of two or three, some of them with a nucleus stained red or blue These are young epithelial cells, and are thicker than the squames or placoids Transition forms exist between them and the clear squames During a maximum distension of the lung they yield and become flattened out In a well-distended alveolus, narrow angular apertures, especially in the cement lines, are to be seen These are the so-called *pseudostomata*, or small apertures which lead into the lymph-canalicular system of the alveolar wall (*Indicate the squames and small granular cells in* Pl XI, Fig 4)

BLOOD-VESSELS OF THE LUNGS

The branches of the pulmonary artery break up into capillaries, which are distributed on the walls of the air-vesicles At the root of the lung the branches of the pulmonary artery and vein and bronchus (p 55) are found together,—the bronchus in the middle, with a blood-vessel on each side of it,—within the lung the pulmonary vein pursues a separate course The *bronchial arteries* enter the lung at its root, and run in the adventitia of the large bronchi, which they supply with blood Sections of them will be found near the nerve-trunks at the root of the lung (p 55)

PREPARATION—Make sections of a lung whose blood-vessels—pulmonary artery, or vein, or both—have been injected with a carmine gelatine (p li) or Prussian blue (p li) mass, and mount them in dammar

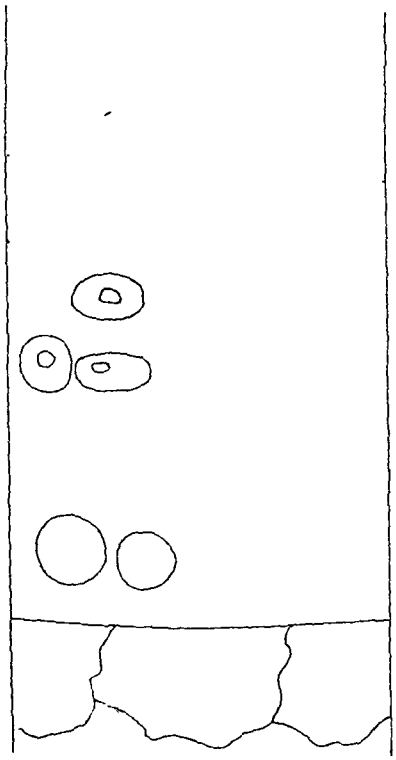
EXAMINATION (L)—Notice the extremely dense plexus of capillaries on the walls of each air-vesicle Trace a small arteriole from a large branch of the pulmonary artery, and note that it may supply two or three adjacent alveoli, while the efferent vein usually passes off at the other side (*Indicate the arrangement of the capillaries in* Pl XI, Fig 5)

(H) Study the capillaries, note the dense network of more or less wavy capillaries, and especially on a septum between two adjacent alveoli A twisted capillary may be seen lying at one time in one alveolus, at another in the adjoining alveoli The space between the capillaries varies according as the lung has been kept distended or not In preparations which have been placed in alcohol, the air-vesicles shrink considerably, and hence the alveolar capillaries appear relatively close to each other The large arterial and venous branches lie in the interlobular septa, which are continuous with the adventitia

LYMPHATICS OF THE LUNG

These are very numerous, and form three systems —(1) the *subpleural* lymphatics occur in the deep layer of the pleura, and communicate with the pleural cavity on the one hand, and have a direct connection with the lymphatic canalicular system, which lies in the alveolar wall, and which communicates with the pseudo-stomata of the air-vesicles Branches of this system pass through the interlobular septa to reach (2) the *perivascular* lymphatics

PLATE XI LUNG



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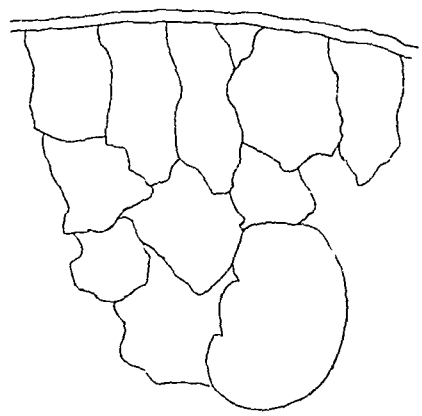

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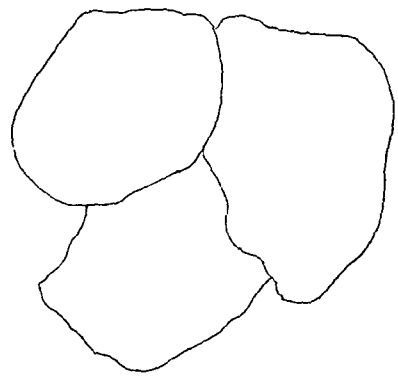

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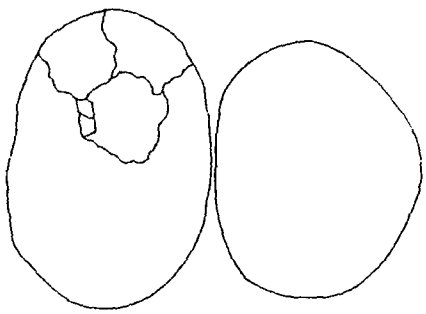

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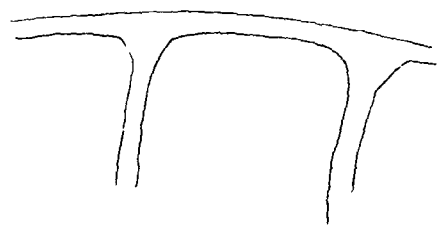

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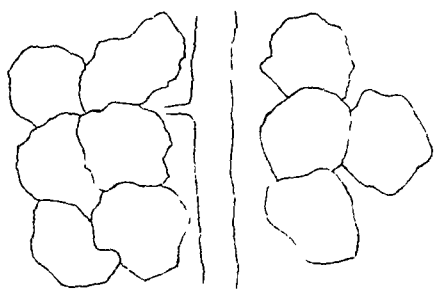

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which accompany the branches of the pulmonary artery and vein, (3) the *peribronchial* lymphatics which occur in the adventitia of the bronchi, communicate freely with the perivascular lymphatics, and run towards the bronchial glands In the walls of the bronchi of the cat large masses of adenoid tissue or lymph-cords are easily found (Klein, Sanderson, and Stirling) They are not nearly so abundant in the human lung

PREPARATION —The lymphatics of the bronchi may be injected by the puncture method, but a good idea of their arrangement may be obtained by the student from the lungs of a coal-miner who has suffered from anthracosis The particles of soot and charcoal are carried into the lymphatics, which therefore appear black Make a vertical section through the pleura and lung-tissue of such a lung, which has been hardened in alcohol, and mount it in Farrant's solution

EXAMINATION (L) —Observe the black pigment distributed in the lung Note that the superficial layer of the pleura has no pigment in it, though the deep layer is markedly pigmented Valves prevent the passage of the pigment from the deep into the superficial lymphatics of the pleura Trace from the pleura inwards strands of pigment in the interlobular septa, in the outer coats of the arteries and bronchi, and it may be seen here and there between the air-vesicles, and also in the adventitia of the bronchi and arteries (Compare Klein's 'Anatomy of the Lymphatic System,' pt II, 1875)

The **Nerves of the Lung** are very numerous, and enter it with the bronchi As already indicated, they may be seen in a section of the root of the lung They lie outside the cartilages in the bronchial adventitia, and are accompanied by branches of the bronchial arteries Five or six strands of nerve-fibres may be found in one section Many ganglia (Remak, Klein, and Stirling) are found in their course, and are often seen in section They can be easily isolated from a bronchus with the aid of a dissecting microscope (p xxxv)

The Nerves of the Trachea —I have recently discovered a large number of ganglia intercalated in the course of the nerves, lying just outside the posterior muscular wall of the trachea Some of them may be seen in sections of the trachea

ALIMENTARY CANAL, AND THE GLANDS CONNECTED WITH IT

THE LIPS

PREPARATION—Place small pieces of the lips of a dog or cat in chromic acid and spirit mixture for two weeks, and, when they are hardened, make vertical sections of them in the ordinary way Stain a section with picrocarmine, and mount it in Farrant's solution

EXAMINATION (L)—Observe the hair-follicles on the outer surface of the lip, and the inner surface covered by stratified squamous epithelium, and if the animal's skin was pigmented the deeper layers of the epithelium contain black pigment or melanin Between these two surfaces observe the transverse sections of the orbicularis oris muscle, its muscular bundles surrounded by connective tissue, continuous with the connective tissue of the rest of the lip All the connective tissue is red, the glands and muscle yellowish, and their nuclei deep red Numerous sections of blood-vessels and nerves are seen (H) Place the stratified epithelium in the field of the microscope and examine it Observe the epithelium, and study particularly the prickle-cells and the pigment in the cells of the deeper layers The various layers of a hair-follicle are easily made out, and the sebaceous glands opening into them are easily studied (compare hair-follicles, p 93)

TONGUE

The muscular substance of the tongue is enclosed in a mucous membrane, covered with stratified epithelium The mucous membrane on the back and sides of the tongue is provided with papillæ—filiform, fungiform, and circumvalate—and on these are small secondary papillæ There is a thin sub-mucous layer, whose connective tissue is continuous with that surrounding the muscular substance The muscular substance consists of longitudinal, transverse, and vertical bundles of striated muscle In the posterior part of the dorsum of the tongue are to be found mucous and serous glands and adenoid tissue, and on the sides of the tongue the papillæ foliatæ, in which lie the taste-bulbs

PREPARATION—Harden the tongue of a cat, and also a part of the human tongue, in chromic acid and spirit mixture for two weeks, and make transverse sections Stain one with logwood and mount it in dammar, and stain another with picrocarmine and mount it in Farrant's solution

EXAMINATION (L)—Observe the papillæ, confined to the dorsum of the tongue, beset with secondary papillæ, and covered with epithelium, beneath these the scanty sub-mucous coat, containing sections of blood-vessels In the middle of the muscular substance a vertically placed septum, and muscular fibres running transversely out from it Observe muscular

fibres disposed vertically, and terminating above in tendons continuous with the connective tissue of the mucous membrane Immediately under the mucous membrane of the dorsum notice the transverse sections of longitudinal muscular fibres Between the muscular fibres many fat-cells are seen, and excellent sections of nerves, and the branches of the ranine artery are found in the lower part (*Indicate the general arrangement of the parts in one half of* Pl XII, Fig 1) (H) Study the papillæ, and the epithelium covering them The upper layers of the epithelium are often horny, especially in animals Notice the transverse sections of muscular fibres—polygonal in shape—with the nuclei placed just under the sarcolemma (Pl XII, Fig 2)

POSTERIOR PART OF THE TONGUE—Prepare this just as the anterior part, and make vertical sections Here the mucous membrane is much thicker and looser, and contains many secreting glands, lymphatic follicles, and diffuse adenoid tissue Stain a section with logwood and mount it in dammar

EXAMINATION (L)—Perhaps the section may have passed through a circumvalate papilla Study specially the *secreting glands*, which are of two kinds, *mucous* and *serous* (v Ebner) The *mucous* (L and H) glands are like similar glands in the mouth and œsophagus (p 65) Each gland consists of a duct and a secreting part The duct passes vertically and opens on the free surface, often with a funnel-shaped mouth It has a wide lumen, and a membrana propria with oval nuclei, lined by a layer of columnar epithelium The duct branches and terminates in the secretory part, which consists of more or less convoluted tubes, so that each gland is a compound tubular gland The cells lining the secretory alveoli consist of a single layer of low columnar epithelium, and their appearance is very characteristic, especially when they are distended They are clear and transparent, with a thin oval nucleus pushed to one side They contain a delicate network of fibrils distended with 'mucigen,' which yields mucin During a state of exhaustion these cells are more 'granular' The *serous glands* (L and H) always bear a relation to the taste-bulbs, and these ducts open into grooves which contain these bulbs The secreting epithelium is columnar with a spherical nucleus, and the protoplasm is very granular, due to the existence of a dense fibrillar network This markedly granular character at once distinguishes them from the clear transparent cells of the mucous glands They seem to secrete a watery fluid In the same section patches of adenoid tissue may be found

Double-Staining of the Tongue with Logwood and Iodine Green—Stain a vertical section of the posterior part of the tongue lightly in logwood, wash it thoroughly, and then stain it slightly in a weak watery solution of iodine green (p xlv), mount in dammar The striking feature is that the acini of the mucous glands are stained of a bright green, while the epithelium in the efferent duct is of a logwood tint A high power reveals the nuclei stained with logwood The serous glands do not take on any of the green pigment, so that the two kinds of glands stand out in striking contrast Make a similar preparation with **picrocarmine and iodine green** as indicated for the small intestine (p 69), mount in dammar All the muscle will be yellowish-red, the connective tissue deep red, and the adenoid tissue and mucous glands bright green

Blood-vessels of the Tongue—Mount a vertical section of an injected tongue in dammar The blood-vessels present nothing peculiar Trace branches into the papillæ If the papilla be simple it contains a single capillary loop, if compound, each secondary papilla contains a similar loop (*Fill in the blood-vessels in one half of* Pl XII, Fig 1)

Nerves of the Tongue—Many sections of the hypoglossal nerve will be found near the

ranine artery in any of the above sections In the course of the lingual and glosso-pharyngeal nerves, not unfrequently fine ganglia are seen I have often seen them in sections, and isolated them from these nerves (Remak, Kolliker, Stirling)

TASTE-BULBS —The papillæ foliatæ on the sides of the back part of the rabbit's tongue are hardened in the same way as the tongue Make vertical sections through the leaflet-like papillæ After staining them with logwood, mount in dammar They are also found on the circumvalate papillæ, but the papillæ foliatæ are preferable They are also found on the posterior surface of the epiglottis

EXAMINATION (L) —Observe the flattened papillæ lying side by side, and on each side of a papilla the four oval or flasklike taste-bulbs At the base of the papillæ the ducts of serous glands (p 61) may be met with (Pl XII, Fig 3)

(H) Study a taste-bulb made up of narrow epithelial cells arranged like the staves of a barrel, investing modified epithelial cells, each provided with a fine process, which projects through an opening at the upper part of the investing cells (Pl XII, Fig 4)

The 'inner' and 'outer' cells may easily be isolated by teasing out a small piece of a papilla, hardened in a quarter per cent osmic acid solution

TOOTH

UNSOFTENED TOOTH

PREPARATION —The same as that employed for dense, dry bone (p 33) It is better to purchase a section ready-made than to spend time in grinding down a section

EXAMINATION of a vertical section (L) —Observe the dentine or ivory forming the great mass of the tooth and surrounding the pulp-cavity in the centre, the enamel covering the crown and sides of the dentine and the crusta petrosa or cement, a layer of bone without Haversian canals covering the fang In the enamel are a number of concentric dark lines—'contour lines'—running across it In the dentine, note the wavy lines and their varying direction—the dentinal tubules They stand out clearly because they are filled with air Arched incremental lines may be seen running across the dentine

(H) **Dentine** —In the homogeneous matrix the dentinal tubules, which open by one end into the pulp-cavity and run out with a wavy course through the dentine, dividing dichotomously, and giving off many anastomosing lateral branches, end in the outer part of the dentine, either in loops, or they open into irregular spaces—the interglobular spaces

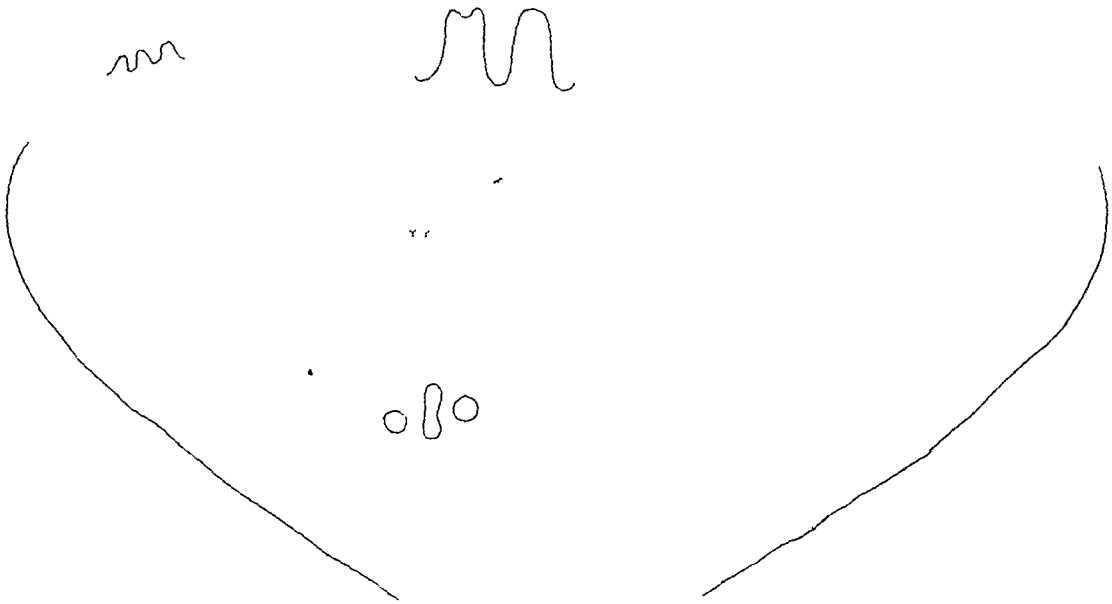
Cement or Crusta Petrosa —Like bone but without Haversian canals Some of the lacunæ may open into the interglobular spaces

Enamel —Observe the transversely striated polyhedral prisms set in groups on the dentine *If cut transversely, they are seen to be hexagonal* Not unfrequently cracks are seen in the enamel where it rests on the dentine, and these cracks appear black from being filled with air

SOFTENED TOOTH

PREPARATION —Take the lower jaw of a cat or dog, and carefully free it from its muscles, but retain the periosteum Cut it in pieces of an inch long with a saw, and place the pieces in

PLATE XII TONGUE & TOOTH



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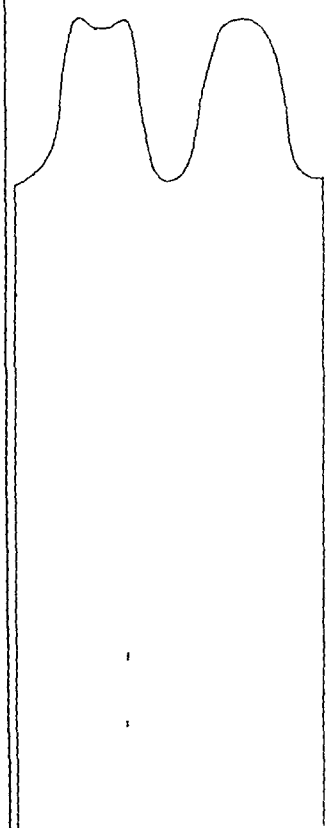

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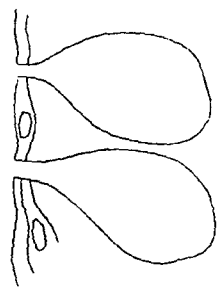

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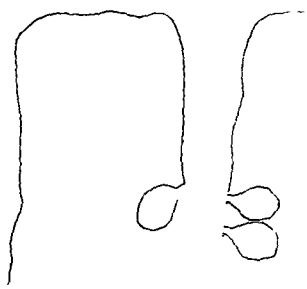

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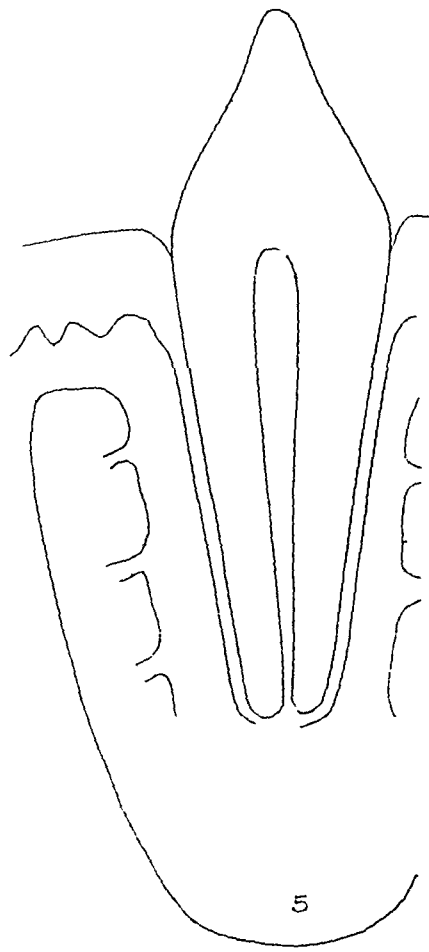

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a large amount of chromic acid and nitric acid fluid until they are sufficiently decalcified, which may be ascertained by piercing them with a needle Decalcification takes place in two to three weeks if the fluid be frequently renewed Wash the pieces frequently in water to remove the surplus acid, and then transfer them to spirit until sections are required Make a number of vertical sections through the jaw, and the tooth implanted therein Make a number of sections across the long axis of a tooth and keep them till they are required In the process of softening, the enamel disappears, owing to the small amount of organic matter present Stain a vertical section and a transverse section in picrocarmine, and mount each in Farrant's solution

Vertical section of a softened tooth **EXAMINATION** (L)—Observe the absence of enamel, though the general distribution of the parts of the tooth is the same as in the unsoftened tooth Notice the tooth planted in the alveolus, which is lined by a membrane stained red—the *periodontal membrane*—which serves as a periosteum for the bony wall of the alveolus Trace its continuity with the fibrous tissue of the mucous membrane of the gum In the lower jaw-bone, observe the medullary cavity with branches of a nerve and blood-vessels They are very apt to fall out, however The pulp-cavity is seen to contain the pulp (*Indicate the general arrangement of the parts in* Pl XII, Fig 5)

(H) Observe the dentinal tubules, which are, however, less distinct than in the dry tooth, the matrix is stained yellow Here and there transverse sections of the tubules may be seen, they appear as fine dots in a homogeneous matrix Notice the crusta petrosa with its bone-corpuscles In the pulp-cavity traces of blood-vessels, delicate connective tissue, and, it may be, a layer of columnar cells—the *odontoblasts*—may be found lying on the dentine Place a similar section in one per cent osmic acid for four hours, mount it in Farrant's solution, and examine It shows the same details more clearly

Transverse section of a softened tooth **EXAMINATION** (L and H)—Observe the pulp-cavity and the dentinal tubules radiating from it, and many of them cut transversely

DEVELOPING TOOTH

PREPARATION—Place the head of a new-born rat, kitten, or puppy in a large quantity of Muller's fluid for two days, and then into a sixth per cent solution of chromic acid for two days At the end of another two days, substitute for it the chromic and nitric acid mixture (p xxxiii) until complete decalcification takes place Make vertical sections through the jaw, and pass them through a five per cent solution of sodic bicarbonate, to remove all trace of acid Stain a section with picrocarmine, another must be left in one per cent osmic acid for several hours, and mount both in Farrant's solution

EXAMINATION (L)—Observe the general shape of a tooth-germ still imbedded in the gum Note the dentine and, it may be, the cap of enamel, inside the dentine, the large mass of tissue which becomes the pulp

(H) Lining the dentine, observe a layer of columnar cells—the odontoblasts—which give off fibres—the fibres of Tomes—which pass into the dentinal tubules

SALIVARY GLANDS

The salivary glands do not all possess the same structure, nor do corresponding glands in different animals exactly resemble each other, hence the necessity for examining the various glands of different animals There are *three* kinds of salivary glands, which differ in structure and in the nature of their secretion —

(*a*) **True Salivary Glands**—as the sub-maxillary gland of rabbits and the parotid of man and some mammals

(*b*) **True Mucous Glands**—as the sub-maxillary glands of cat and dog, and the human sublingual

(*c*) **Muco-salivary Glands**—as the sub-maxillary gland of man and guinea-pig

They are all compound tubular glands, made up of branched tubes of varying lengths The ducts in all are practically the same They are lobulated and supported by a connective-tissue framework The nature of the lumen, and the characters of the epithelium lining the alveoli, constitute the chief differences between them In the **true salivary glands** (*a*) the lumen of the alveoli is small, and they are lined by a single layer of low columnar epithelial cells, each with a nucleus near its attached end These cells are markedly 'granular,' and, as Heidenhain has shown, they vary in appearance during rest and when they are secreting In **true mucous glands** (*b*) there are two kinds of cells—one, mucous cells, moderately tall, clear columnar epithelial cells, each with a small nucleus pushed quite out to the membrana propria of the alveolus They are in fact not unlike goblet-cells, and contain a fine network, whose meshes are filled with mucigen (Heidenhain), this yields mucin, which stains deeply with logwood Outside these, at various parts between them and the alveolar walls, are the 'crescents' (Gianuzzi), or parietal granular cells They are small nucleated polyhedral cells In **muco-salivary glands** (*c*) are found alveoli, which present the characters of true salivary glands, and others exactly like those of mucous glands

PREPARATION (*a*) —Place the sub-maxillary glands of a dog and rabbit in absolute alcohol for forty-eight hours Make transverse sections Stain with logwood, and mount one in dammar and another in Farrant's solution This is the best method for a general survey

(*b*) Harden another gland in chromic acid and spirit mixture for a week, and stain sections with logwood and mount in Farrant's solution

(*c*) Small pieces, the size of half a pea, may be hardened in a quarter per cent osmic acid, and small pieces teased in glycerine, or sections may be made and mounted in glycerine It brings out the 'crescents' very clearly in the mucous glands

Sub-maxillary Gland of a Dog **EXAMINATION** (L) —Observe the lobulated character of the section, the fibrous capsule sending septa into the gland, and containing blood-vessels, lymphatics, and nerves Notice the alveoli or gland-substance cut in every direction, and in the interlobular septa here and there a large duct—lobar—cut into (*H*) Study a transverse section of a large lobar duct Notice the columnar epithelium lining it, with vertical lines due to the longitudinal arrangement of the fibrils, and the nucleus placed about the middle of the cell Within a lobule may be found sections of an *intra-lobular duct*, which have a small lumen lined by columnar epithelium

Study an Alveolus —Observe the secretory epithelium lining it The epithelium may be

quite clear, with its small nucleus near the alveolar wall Study the lunate cells or crescents of Gianuzzi (*Indicate the alveoli, the cells lining them, and a lobar duct in* Pl XIII, Fig 1)

Sub-maxillary Gland of a Rabbit **EXAMINATION** (L) — Observe the same general characters

(H) **Study an Alveolus** —Observe the small lumen and the single layer of low columnar cells lining it Compare it with the above description

TONSILS

The tonsils are folds of mucous membrane containing a large number of lymph-follicles like those composing a Peyer's patch (p 69) The mucous membrane is folded so as to eave pits or depressions visible on the surface The epithelium covering them is stratified, and under it is a connective-tissue mucosa, which contains the closely aggregated masses of adenoid tissue

PREPARATION —Harden the tonsils for two weeks in chromic acid and spirit mixture, or in Muller's fluid for three weeks Make transverse sections, stain with logwood, and mount in dammar

EXAMINATION (L)—Note the stratified epithelium, and under it the oval or rounded masses of adenoid tissue, which sometimes penetrate into the epithelium

(H) The ordinary structure of adenoid tissue

ŒSOPHAGUS

PREPARATION —Slightly distend the œsophagus of a dog with the chromic acid and spirit mixture, and keep it distended by placing a ligature on its upper and lower ends Suspend it in a large quantity of the same fluid until it is 'fixed'—*i e* for twenty-four hours—then cut it into pieces one inch long and continue the hardening for a week or ten days Make transverse sections through the upper and lower parts of the œsophagus Stain them with picrocarmine, and mount in Farrant's solution

Transverse section of the Œsophagus **EXAMINATION** (L)—Observe the mucous coat lined by stratified epithelium with well-marked mucous glands opening on its surface The mucous membrane is beset with small papillæ In the deeper part of the mucous coat observe the cut ends of the muscularis mucosæ, composed of non-striped muscular tissue arranged in bundles In the sub-mucous coat observe the alveoli of the mucous glands and trace a duct to the surface Observe the muscular coat—the outer or longitudinal layer—cut transversely, and the circular fibres inside it These contain much striped muscle in the upper part of the œsophagus

(H) Study the stratified epithelium and the mucous glands, which are exactly like those already described in the mouth (p 61) A preparation mounted in dammar shows their 'mucous' characters admirably

Nerves of the Œsophagus —The nerve-terminations are best studied in the œsophagus of a rabbit by means of the lemon-juice and gold method The process is described at p 71, in connection with the small intestine

STOMACH

PREPARATION—Open the stomach of a cat, dog, or rabbit, and wash away the layer of mucus lining it with a stream of salt solution

(*a*) Harden small pieces of the pyloric and cardiac ends and middle of the stomach for two weeks in the chromic acid and spirit fluid (p xxxi) Complete the hardening in spirit Make vertical sections of each piece Stain sections with logwood, and mount them in dammar, and others with picrocarmine, and mount them in Farrant's solution

(*b*) After washing out a stomach of any of the above animals with salt solution, distend it with and place it in absolute alcohol—or merely small pieces may be hardened in absolute alcohol These show gland-structure well, and stain readily

(*c*) Very small pieces, not larger than a pea, are to be hardened in a quarter per cent osmic acid for twenty-four hours Make sections and mount them in Farrant's solution

CARDIAC END OF STOMACH

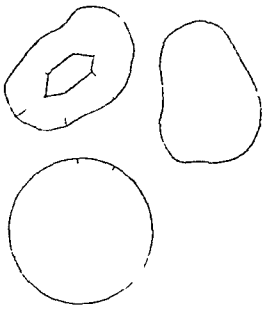
Vertical Section (Logwood and dammar) **EXAMINATION** (L)—Observe the *mucous coat* with its gastric glands placed vertically Trace a gland and note whether it branches below or not The bases of the gland rest on a small amount of connective tissue, which sends up processes between the glands Immediately outside this lies the muscularis mucosæ, which consists of two layers—longitudinal and circular—and sends processes upwards between the glands (*Indicate this general arrangement in* Pl XIII, Fig 2)

The *sub-mucous coat* composed of connective tissue containing blood and lymph-vessels, fat-cells, and nerves The *muscular coat*, consisting of two coats, with perhaps a third The appearance of these coats varies according to the plane of their section, and outside this, the *serous* layer, or peritoneum If the stomach of the cat be selected, a clear band of condensed connective tissue will be observed lying immediately above the muscularis mucosæ This preparation shows well the passage of the muscularis mucosæ fibres upwards between the glands

To see the finer details of structure study the preparation mounted in Farrant's solution (L) Observe the same general arrangement as before (H) Study a *peptic gland* Note the clear, tall, narrow, columnar epithelium lining the stomach, and continued some distance into it The nuclei of the cells are placed near their attached ends The cells appear in some cases to be open at their free ends, especially if the animal be killed during digestion, and are, in fact, mucus-secreting goblet-cells Note that the lower part of the cell is always granular, even though the upper two-thirds be quite transparent In the middle and lower part of the gland note the coarsely granular nucleated outer, ovoid, or peptic cells, which frequently cause a bulging on the side of the gland, and, internal to these, small ill-defined inner or central cells, best seen at the lower part of the gland A small narrow lumen may be detected in the gland The cells most deeply stained are the ovoid cells Study the muscularis mucosæ, and note that it consists of two—sometimes three—layers of non-striped muscle, and trace processes from it upwards between the gland

Transverse sections of the glands are to be met with in the same section They are usually in groups of four or five, and separated by a little connective tissue and a few non-

PLATE XIII SUB-MAXILLARY GLAND & STOMACH



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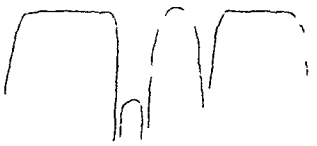

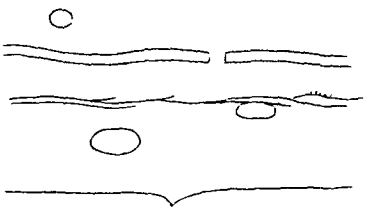

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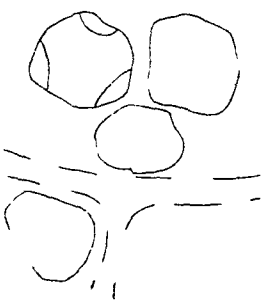

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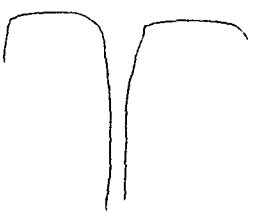

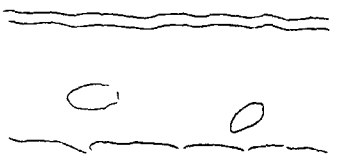

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striped muscular fibres Observe the relation of the outer and inner cells, and notice the small lumen of the gland (*Indicate the appearance of transverse sections of the tubes in* Pl XIII, Fig 4.)

Aniline Blue Stain a section with a watery solution of aniline blue Mount it in glycerine or dammar The peptic cells are most deeply stained

PYLORIC END OF STOMACH

EXAMINATION —Observe the mucous coat, containing glands (mucous) which branch frequently, and do not contain any large ovoid cells The gland-ducts are relatively long, and two or three tubes open into a short neck The gland-tube is lined by the same kind of epithelium throughout Notice transverse and oblique sections of the gland at the lower part of the mucous membrane Here and there, near the base of the gland, detached patches of adenoid tissue may be seen (*Indicate the general arrangement in* Pl XIII, Fig 5)

Take another section, stain it with carmine, and mount in Farrant's solution Notice the same general arrangement as above Observe that the glands are lined throughout with columnar epithelium Study a transverse section, and note the basement-membrane of a gland Notice a few leucocytes lying near the bases of the glands

Isolation of the Gastric Glands **PREPARATION** — Place small pieces of the mucous membrane of the stomach of a rabbit in five per cent ammonium chromate for three days Wash away the colouring matter from a small piece of the membrane, and place it in picro-carmine solution for twenty-four hours, tease a small piece in glycerine This method shows the shape and general characters of the glands, and their cellular contents, the ovoid cells being specially distinct The nucleus is stained of a bright red, and a beautiful intra-cellular plexus of fibrils is revealed Note also the basement-membranes of the gland-tubes (*Indicate the cells in the gland-tube in* Pl XIII, Fig 3)

The *osmic acid preparations* show the peptic cells very well They are more deeply stained than the other cells, and hence they stand out more clearly

Blood-vessels of the Stomach **PREPARATION** —Inject the blood-vessels of the stomach of a cat or dog through the gastro-duodenal artery with a carmine gelatine mass (p li), and place the organ in alcohol Make vertical sections and stain them with logwood and mount in dammar If a blue mass be used stain the sections with carmine

EXAMINATION (L) —Observe the vascularity of the mucous membrane, sections of the large vessels will be found in the submucous coat, and from these capillaries proceed upwards between the glands, and form a capillary plexus round them The veins rise near the surface, and run downwards towards the submucous coat

DUODENUM SMALL INTESTINE

PREPARATION —Wash out the contents of the duodenum of a dog or cat with a stream of salt solution, and place pieces one inch long in chromic and spirit fluid for two weeks If the duodenum of a pig can be obtained, it is to be preferred Place similar pieces of the small intestine some with, others without a Peyer's patch, in the same hardening fluid for the same time Complete the hardening in alcohol Make transverse sections Stain a section of the

duodenum with logwood, and mount it in dammar Stain sections of the intestine—with and without a Peyer's patch—with logwood, mount one in dammar, and another in Farrant's solution Place sections of the small intestine in a quarter per cent solution of osmic acid for twenty-four hours, and mount a very thin section in Farrant's solution

Vertical Section of the Stomach and Duodenum—Make a vertical section through the pyloric valve, so as to include a part of the stomach and the upper part of the duodenum Stain a section in logwood, and mount it in dammar

EXAMINATION (L)—Observe the gastric mucous membrane with its mucous glands, and trace a gradual transition from these into the compound tubular and more branched glands of the duodenum—Brunner's glands, which present exactly the same structure as the mucous glands of the stomach, with this difference, that their duct is larger, and each duct has a greater number of long branched tubes attached to it The alveoli lie in the submucous layer outside the muscularis mucosæ, and from these the duct lined by columnar cells pierces the muscularis mucosæ, and ascends between the crypts of Lieberkuhn to open on the surface A few villi are seen on the duodenal mucous membrane At the line of junction of the mucous membrane of the stomach and duodenum the muscularis mucosæ is absent

SMALL INTESTINE

Transverse Section (Logwood and dammar) **EXAMINATION** (L)—*Mucous coat*—Observe the conical pointed villi, some of them may be contracted and show the epithelial covering thrown into folds Each *villus* consists of a central core covered with columnar epithelial cells, with their nuclei placed near the attached end of each cell Beneath the villi the mass of the mucous membrane is made up of *Lieberkuhn's follicles*, consisting of simple tubular glands lined by epithelium Note the adenoid tissue between the bases of the glands, and outside this the *muscularis mucosæ* The *submucous coat* consists of connective tissue containing blood-vessels and nerves *Muscular coat*—Note the thick inner circular layer, which will appear different according as the section has been made across or parallel to the long axis of the gut, if the latter, note that the muscular fibres are arranged in blocks separated and surrounded by connective tissue, outside this the narrower longitudinal layer, and outside all the *serous* coat This specimen does best for a general study of the relation of parts Select a villus and examine it with (H) Notice the long fusiform nuclei in that part of the core of the villus, next the central lacteal vessel They are the nuclei of non-striped muscular fibres, which pass from the muscularis mucosæ into the villi (*Indicate the general arrangement in one-half of* Pl XIV, Fig 1)

Examine now a similar preparation stained with logwood, but mounted in Farrant's solution Observe the same relation of parts as described above Fix a villus in the field, and observe (H) the epithelial investment, consisting of columnar nucleated cells with a clear hem containing vertical striæ (compare p 10, on epithelium) Observe goblet-cells scattered between the epithelial cells and study them (*Indicate the covering and structure of a villus in* Pl XIV, Fig 2) Search for these cells seen *en face* In the substance of the villus observe the adenoid tissue of which it consists, with nuclei in its meshes, and perhaps a lacteal vessel in its centre

Lieberkuhn's Glands Study one Observe its test-tube shape, and the low nucleated columnar epithelium lining it At their bases and outside them observe adenoid tissue Study the two layers of the muscularis mucosæ

Submucous coat—Observe its blood-vessels, and perhaps nerve-ganglia (*Meissner's* plexus), and perhaps a solitary gland

Muscular Coat—Observe the characters of non-striped muscle cut transversely and longitudinally

PEYER'S PATCH

Transverse Section (Logwood and dammar) **EXAMINATION** (L)—Observe the masses of adenoid tissue in the submucous coat Their conical points project upwards into the gut, and are covered by epithelium, but no villi exist over them

Treble-staining of the Small Intestine PREPARATION—Take one of the transverse sections of the small intestine, containing a Peyer's patch Stain it with picrocarmine, wash it lightly in water slightly acidulated with acetic acid Stain it now with solution of iodine green (p xlv) until it becomes slightly green, wash it quickly in water, and mount it in dammar, taking care that it does not remain too long in the alcohol

EXAMINATION (L)—This is an exquisitely beautiful preparation All the glands, and especially Peyer's patches, are green, all connective tissue is red, and so the submucous layer stands out bright red The muscularis mucosæ and muscular coats are yellow Observe the interruption of the muscularis mucosæ, where the adenoid follicles reach the surface A thin layer of connective tissue lies superficial to the muscularis mucosæ Such preparations keep a long time

In the preparations steeped in osmic acid, after hardening with the chromic acid mixture, the various details are seen with exquisite clearness

Blood-vessels of the Small Intestine—Make transverse sections of the small intestine of a cat or dog whose blood-vessels have been filled with a carmine and gelatine mass Mount in dammar It is easy to inject the intestine from the superior mesenteric artery

EXAMINATION (L)—Observe the villi, and note their vascularity Usually one artery ascends on one side, and splits into capillaries, which are distributed closely under the epithelium The vein descends on the opposite side of the villus Note the rich plexus of capillaries surrounding Lieberkuhn's glands, and the large arterial trunks in the submucous coat Study the distribution of the blood-vessels in the muscular coat, and observe that it is not so vascular as the mucous coat (*Indicate the arrangement of the blood-vessels in one half of* Pl XIV, Fig 1)

Injected Villi seen from above—Mount in dammar a small piece of the small intestine of a rabbit whose blood-vessels have been injected with a carmine and gelatine mass Place the section in the slide so that the villous surface is uppermost

EXAMINATION (L)—Observe the leaflet-like injected villi directed towards the observer, and by focussing through the thickness of the preparation the course and distribution of the larger arterial trunks in the submucous coat can be clearly made out

Lymphatics or Lacteals of the Small Intestine—The origin of the lacteal within the central part of a villus as a blind tube has already been noted (p 68), but the branches of the lymphatics in the walls of the gut can easily be studied, after injection with Berlin-blue, by the puncture method, an excellent view of their arrangement will be obtained in the preparation of the intestine treated with silver nitrate (p 37), to show the cement-substance of muscle This will be referred to again under lymphatics (p 76)

VERMIFORM APPENDIX

PREPARATION — Inject the walls of the vermiform appendix of a rabbit with a two per cent watery solution of Berlin-blue, which readily passes into the lymphatics Harden in alcohol, and make transverse sections Stain them with picrocarmine, and mount in Farrant's solution

EXAMINATION (L) —Observe the masses of adenoid tissue, stained red, divided into an inner and an outer set, and round each the lymph-paths filled with Berlin-blue The masses of adenoid tissue are exactly like Peyer's patches, and each arc is partially surrounded by a lymph-stream, as indicated by the blue injection, none of which passes into the interior This space represents a *lymph-sinus*, so that the masses of adenoid tissue are partially suspended in a lymph-stream Exactly the same relation obtains in a Peyer's patch (*Indicate these appearances in* Pl XIV, Fig 5)

LARGE INTESTINE

PREPARATION —After washing out the large intestine of a dog or cat, cut it into pieces one inch square and harden them in chromic acid and spirit fluid for two weeks and complete the hardening in spirit Make transverse sections Stain with logwood, and mount one in dammar, and another in Farrant's solution

Vertical Section of the Large Intestine (Logwood and dammar) **EXAMINATION** (L) — Observe the mucous coat thrown into folds, owing to the contraction of the muscular coat It is devoid of villi Observe the vertically set Lieberkuhn's glands, exactly like those in the small intestine (*Indicate one in* Pl XIV, Fig 3) Some of them are sure to be cut transversely, when they present a honeycomb-like appearance (*Indicate this in* Pl XIV, Fig 4) Observe the sub-mucous and muscular coats, very like those of the small intestine Perhaps a solitary gland may be found in the sub-mucous coat with its apex projecting free into the mucous coat There is a well-marked muscularis mucosæ in the mucous coat, which is pierced by the solitary glands

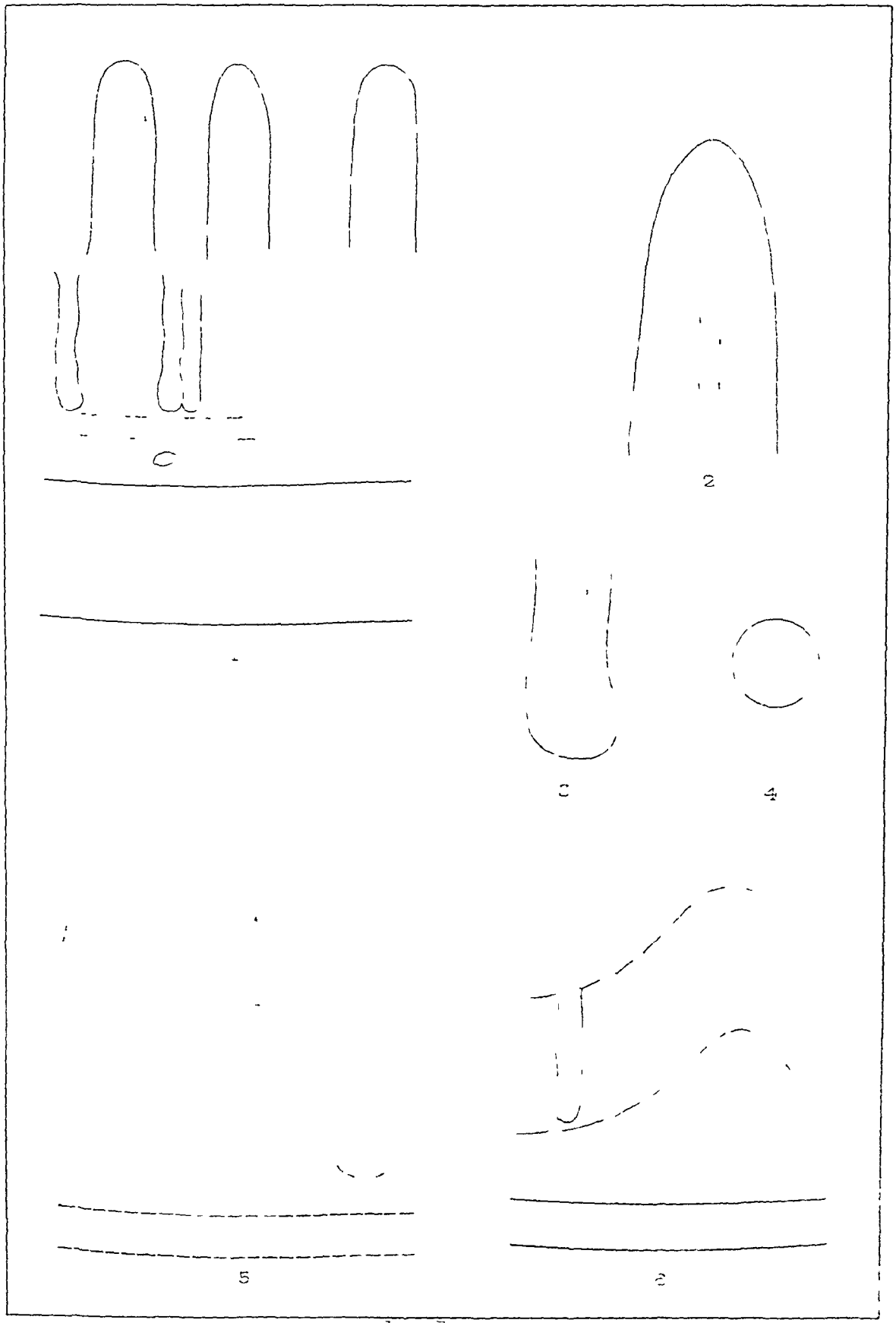
In the preparation mounted in Farrant's solution, examine (H) the glands, both when divided vertically and transversely, and observe the adenoid tissue laden with lymph-corpuscles, between and supporting them

Solitary glands will be found in sections of the large intestine They are simply masses of adenoid tissue placed in the sub-mucous coat They are easily stained by the same methods as directed for Peyer's patches (p 69)

Blood-vessels of the Large Intestine are prepared exactly in the same way as those of the small intestine Several years ago it was customary to use an opaque injection, which gives a beautiful effect when the piece of intestine is mounted upon a dark ground with the mucous surface uppermost, and illuminated by means of a condenser The mouth of each gland is surrounded by a plexus of capillaries, so that the injection presents a regular honeycomb-like appearance

Similar preparations made of the gastric mucous membrane at first sight resemble that of the large intestine The gastric mucosa is recognised by the existence of small openings at the bottom of the depressions, and the latter are not so regular as those in the large intestine

PLATE XIV SMALL & LARGE INTESTINE



NERVE-PLEXUSES OF THE INTESTINE

There are two plexuses of nerves, consisting chiefly of non-medullated nerve-fibres and ganglionic cells In the intestinal walls, one—*plexus myentericus of Meissner*—lies in the submucous coat, the other—*Auerbach's plexus*—lies between the muscular coats

PREPARATION—Auerbach's plexus is best prepared in the following way (*a*) Wash out a loop of perfectly fresh small intestine of a rabbit, and distend it with the juice of a fresh lemon, ligature both ends of the gut and place it, still distended, in lemon-juice for from five to seven minutes Open the ligatures and wash it thoroughly in water, and fill it with a two per cent gold chloride solution, ligature the gut as before, and suspend it for half an hour in a one per cent gold solution Wash it thoroughly, and transfer it to a twenty-four per cent dilution of formic acid, to reduce the gold The preparation must be kept in the dark After reduction, it has a beautiful, rich, reddish-brown colour Wash it thoroughly and keep it in a preservative fluid (p xl) With forceps it is easy to peel off strips of the outer muscular layer, to which Auerbach's plexus adheres Mount in glycerine The separation takes place more easily after maceration for a week in the preservative fluid This is the best method

(*b*) Another method is to use dilute alcohol, as the distending and softening medium, for forty-eight hours, then to peel off the outer muscular coat as directed above Stain it with logwood, and mount in glycerine This gives fairly good results

(*c*) One-twentieth per cent acetic acid may be used in the same way to fill the gut and to macerate it, for thirty hours After maceration and washing it is well to steep the gut for a short time in five per cent solution of sodic bicarbonate, to get rid of the acid Peel off the muscular coat as before, stain it with logwood and mount it in glycerine

EXAMINATION (L)—Observe the wide-meshed plexus of fairly regular form At the nodes, groups of ganglionic cells are to be detected Ganglionic cells are either in groups, or sometimes disposed lengthwise in the flat, band-like nerve-branches Each band has an endothelial sheath (H) The cells are very variable in size, and some of them are multipolar Nerve-branches are given off from this plexus to supply the longitudinal and circular muscular coats, but their mode of termination is unknown

The Plexus of Meissner is prepared in a similar way, and it supplies the muscularis mucosæ, the blood-vessels and glands of the mucous coat Its meshes are much wider and less regular than Auerbach's The nerve-cells are easily seen in it

In vertical sections of a cat's small intestine, hardened in chromic acid and spirit, groups of ganglionic cells may easily be detected in the sub-mucous coat These are the ganglia of Meissner's plexus

THE LIVER

It is well to examine the liver of several animals as they differ in the complexity of structure It is best to begin with a pig's liver, and to pass to that of a rabbit, a dog or cat, and then to the human organ The liver may be hardened in several ways

PREPARATION A **Chromic Acid and Spirit Mixture**—Place pieces of a perfectly fresh liver (pig, rabbit, cat or dog, and man), about half an inch square, in a large quantity of the above fluid Do not wash them As the liver is very vascular, much blood exudes and causes a precipitate in the fluid, which must be changed at the end of twelve hours As soon

as the fluid appears turbid it ought to be changed The hardening will be completed in two to three weeks Wash the tissues well, and transfer them to spirit until they are required

B **Muller's Fluid** —Larger pieces of the liver may be hardened in this way, as this fluid penetrates organs more readily than A It takes, however, longer to harden (four to six weeks)

C **Two per cent Potassic Bichromate**, which acts in the same way, and in the same time, as Muller's fluid

Make sections of the liver of pig, rabbit, cat or dog, and man, by means of a freezing microtome, and see that the sections are thin and made through the fibrous capsule Some of them are to be stained, and, for non-stained preparations, place the sections in one per cent osmic acid for four or five hours This is an excellent method, and sharpens the outlines of all the tissues

1 LIVER OF A PIG

EXAMINATION (L) —Stain a section with logwood, and mount it in dammar Observe the *fibrous capsule*, and note the processes it sends into the organ, where they become continuous with the fibrous sheath which surrounds each lobule Note that the section is mapped out into a number of distinct, more or less polygonal areas of nearly equal size by bands of connective tissue stained blue , these areas are the *lobules*, and each one is completely invested by a fibrous sheath—a continuation of Glisson's capsule Within each of these areas is the true gland-substance, which will be considered in other preparations This preparation gives the student an excellent idea of the lobular character of the liver (*Indicate the lobules in* Pl XV , Fig 1)

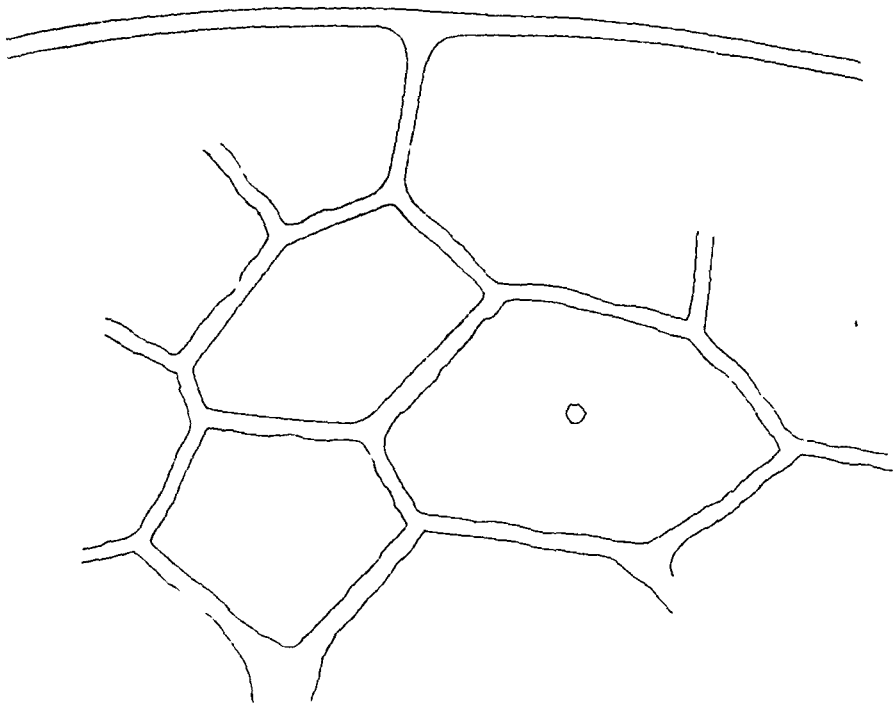
2 LIVER OF A RABBIT

Logwood and dammar Note that the liver-lobules are not nearly so accurately mapped off from each other, but still polygonal areas – sections of the lobules—can be seen mapped off here and there from each other by a very small quantity of connective tissue—*interlobular connective tissue*, or *Glisson's capsule*—stained blue (*Indicate these appearances in* Pl XV , Fig 2)

3 THE LIVER OF A DOG AND OF MAN

These ought to be prepared and studied in the same way In them the outlines of the lobules are even less distinct, owing to the lobules being more or less confluent, due to the small amount of interlobular connective tissue It is, therefore, the amount of interlobular connective tissue which determines the mapping out of one lobule from another Note particularly the thickness of the fibrous capsule, and the small amount of interlobular connective tissue in a normal human liver This is essential, as in some diseases—*e g* cirrhosis —it is increased in amount, and it is necessary to accustom the eye to the normal amount Continue the examination of the **human liver** with (L) Observe the *capsule*, and note its thickness, and that it consists of two layers , the lower one sends very fine processes into the organ between the lobules These processes are better seen in a section where the liver-cells have been pencilled or shaken out, as directed for adenoid tissue (p 29) **Study a lobule** In its centre, if it be cut transversely, observe the *hepatic* or *intra-lobular vein*, and note its size , for it is sometimes dilated in diseased conditions, especially where there is congestion of the lungs and right side of the heart Radiating outwards from this, observe the columns of nucleated liver-cells, united at the outer part of the lobule by transverse branches to form a

PLATE XV LIVER



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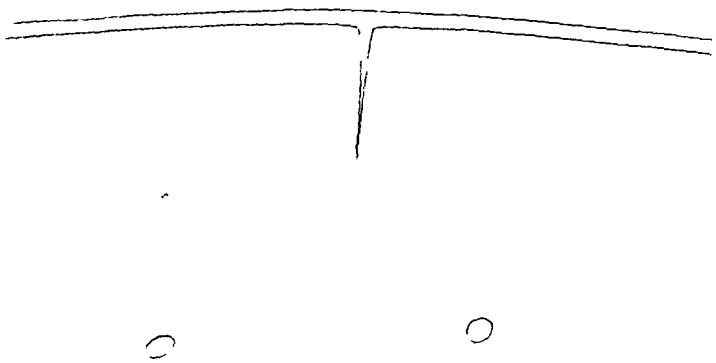

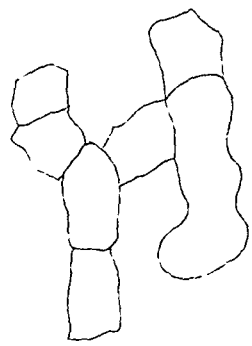

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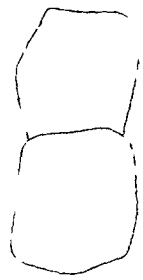

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network. (*Indicate the columns of liver-cells in* Pl XV, Fig 3) The radiate arrangement is best seen in the columns of cells nearest the hepatic vein Between these cell-columns narrow spaces exist, which indicate the position of the intra-lobular blood-capillaries In an uninjected liver the capillaries appear relatively small *Search between lobules* for a transverse section of a branch of the *portal* or *intra-lobular vein*, *hepatic artery*, and *bile-duct*, all of which run together and are surrounded by connective tissue, which constitutes part of Glisson's capsule (*Indicate these structures in* Pl XVI, Fig 2) The channels in which they lie are known as *portal canals* Fix the bile-duct in a field and examine with (H) The bile-duct may be easily distinguished on account of its being lined by low, columnar, almost cubical, epithelium In its wall may be found circularly disposed non-striped muscular fibres, and if a particularly large bile-duct be found, the walls may be seen to contain sections of mucous glands, which secrete mucin and add it to the bile as it passes along the bile-ducts The portal vein is large, and neither it nor the hepatic artery present anything peculiar in their characters Surrounding these is the connective tissue of the capsule of Glisson The tissue is more or less lamellar, the bundles run in various directions, and between them are flattened connective-tissue corpuscles stained blue Note its amount A very small amount of *intra-lobular connective tissue* is to be found, it is best seen around the hepatic veinlet

Study the liver-cells (H)—They are polygonal or cubical cells of nearly the same size, uniformly granular in appearance, are devoid of an envelope, and contain a spherical and deeply-stained nucleus, though some may contain two nuclei, and rare examples are met with without a nucleus The cells at the outer part are usually slightly smaller than the others Note particularly the network formed by these cells, and how they intertwine with the blood-capillaries between them Each cell contains a dense *intra-cellular* and *intra-nuclear plexus* of fibrils, which give it a granular appearance (Pl XV Fig 4) See p 13, where these points are alluded to

Stain similar sections with logwood and picrocarmine, and mount them in Farrant's solution In this medium the finer details can be more carefully studied

Mount an **unstained section**, which has been steeped in osmic acid, in Farrant's solution This is an excellent method for sharpening the outline of the various parts Any oil-globules present within the liver-cells are blackened, osmic acid being an excellent test for the presence of fatty infiltration or degeneration

BLOOD-VESSELS OF THE LIVER

PREPARATION—The blood-vessels of the liver of a rabbit or other animal may be injected either from the portal or hepatic vein, or both It is well to examine a liver whose vessels are injected either with a red or a blue carmine mass, or if desired the portal vein may be injected with a blue mass and the hepatic vein with a red, or *vice versâ* In injecting from the portal vein the mass should flow freely from the hepatic vein, which should then be ligatured, and then the blood-vessels will fill properly One must judge of the amount to be pressed into the vessels by the appearance and colour of the liver-lobules Compare p lii for general directions Harden the organ in two per cent potassic bichromate and then in alcohol, or place the liver at once in alcohol Make sections, and mount them, stained or unstained, in dammar

EXAMINATION (L).—Select a lobule which has been cut transversely, and observe the origin of the hepatic vein in its centre, and at the periphery of the lobule sections of the portal vein Within the lobule, and connecting these two vessels, observe the blood-capillaries

with a distinct radial arrangement near the hepatic vein, though the meshwork becomes more polygonal at its outer part Relatively the hepatic cells appear smaller than in the uninjected specimen Sections of the hepatic vein issuing from within a lobule ought to be looked for A double injection is, of course, very instructive, though the areas of the respective veins may not be accurately mapped out (*Indicate the general arrangement of the blood-vessels in* Pl XVI, Fig 1)

Opaque Injections—It is well to examine by reflected light a section of a liver whose blood-vessels have been injected by an opaque mass—say red in the hepatic vein, and yellow in the portal

BILE-DUCTS

PREPARATION—Inject the fresh and still warm liver of a rabbit or guinea-pig (killed by bleeding) from the bile-duct with a cold, watery, fresh-filtered solution of Prussian blue (p li) This is by no means an easy task The great thing is to use a constant and not too great pressure (p liii) Whenever the outer part of the lobules begins to get blue, stop It is advantageous to inject a red gelatine mass into the portal vein after the injection of the bile-ducts has been completed Place the liver in alcohol for a few hours, then cut it into pieces, and put some in alcohol and others in Muller's fluid for two weeks Make sections in the usual way by freezing

EXAMINATION (L)—In a section of a piece hardened in alcohol and mounted in dammar observe the larger interlobular bile-ducts, filled with a blue mass between the lobules, and from these fine branches can be seen to pass into the lobules, within which they form a polygonal meshwork over and between the hepatic cells (*Indicate the bile-ducts in* Pl XVI, Fig 3)

Stain a section hardened in Muller's fluid with *picrocarmine*, and mount it in Farrant's solution

EXAMINATION (L)—Note the same arrangement as above, only the hepatic cells are better preserved, and their nuclei stained

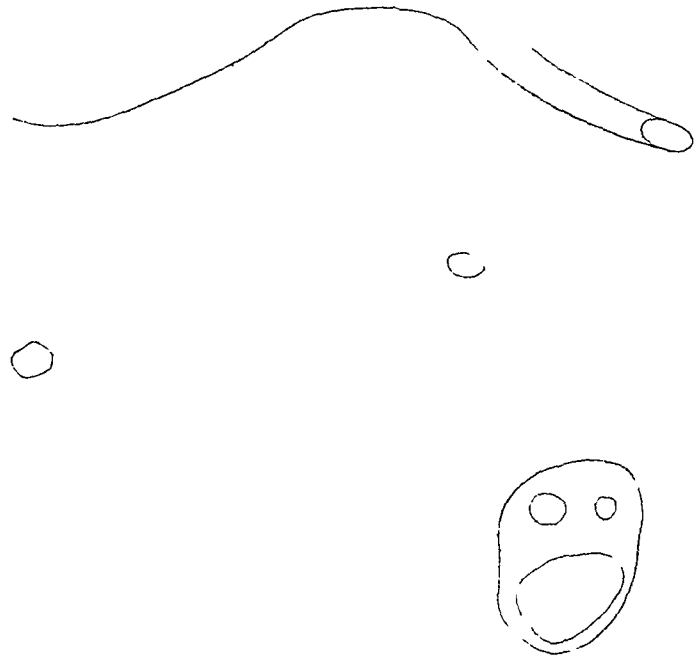
(H) Select a large interlobular bile-duct and observe its lining of low columnar epithelium, and, it may be, sections of a mucous gland in its walls The fusiform nuclei of the non-striped muscle in the wall of the bile-duct are stained red Trace now the *intra-lobular bile-capillaries* or channels, and observe their polygonal shape, *i e*, exactly the shape of the liver-cells, the plexus they form over and between the hepatic cells, whose nuclei are stained red The bile-capillaries are much smaller than the uninjected spaces of the blood-capillaries

In section the blue point indicating a section of a bile-capillary is found in the angle where three or more cells meet They are never found between the liver-cells and blood-capillaries, and are always separated from the capillary blood-stream by a part of one or more liver-cells

Isolated Liver-cells—Scrape the surface of a section of a liver, and diffuse the scraping in salt solution on a slide Compare p 13, where these cells are described Re-examine the osmic acid and picrocarmine preparations of the liver of the rabbit and newt, and in the latter study the intra-cellular plexus

Oil-particles within Liver-cells—These are very common in stall-fed animals—*e g* ox—and are readily recognised by their highly refractive appearance, and by the action of osmic acid, which blackens them in a very short time

Plate XVI Liver & Pancreas



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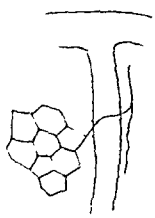

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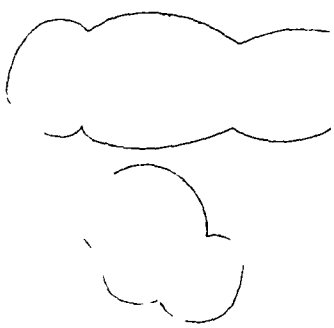

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PANCREAS

The pancreas in many respects resembles the salivary glands, though there are important differences It is lobulated, and has a connective-tissue stroma, blood and lymphatic arrangements like the salivary glands It is a compound tubular gland, and the gland-tissue has lobar and inter-lobular ducts, which terminate by means of an intermediate piece in the alveoli, which consists of wavy, branched, and convoluted tubes, each having a basement-membrane lined by a single layer of columnar or cylindrical cells, whose free ends are sometimes conical The substance of each cell shows a marked division into two zones—the outer (next the membrana propria) is homogeneous, and stains easily and deeply with carmine and logwood—the inner is coarsely granular, and does not stain readily with dyes, and in it lies the spherical nucleus The lumen of the alveolus is small, and so are the alveoli themselves Heidenhain has shown that the appearances and relative sizes of these two zones alter during digestion—*i e* during physiological activity, just like the salivary glands.

PREPARATION (*a*)—Place small pieces of the freshly excised pancreas of a dog in absolute alcohol for forty-eight hours, and then make sections Stain them with carmine, and mount in Farrant's solution, or, better still, with logwood, and mount in dammar

(*b*) Osmic acid preparations are also valuable

EXAMINATION (L)—Observe the lobules, the connective sheath and its septa, perhaps containing the section of a lobar duct Study a lobule, and note an inter-lobular duct, and the alveoli—relatively small—cut in every direction (H) Study an alveolus note the secretory epithelium, with its outer zone stained of a logwood tint, and its inner half granular and unstained This appearance is quite characteristic Scarcely any lumen is observable, and very little interstitial matter separates one secretory cell from another (*Indicate the appearance of the alveoli, and the epithelium lining them, in* Pl XVI, Fig 4)

THE LYMPHATIC SYSTEM

THE **lymphatic vessels** consist of capillaries and trunks of variable size The capillaries consist of single layers of squames with characteristic sinuous edges, and the larger vessels have a structure resembling veins with three coats, but the walls are very much thinner. They have valves in their course

LYMPHATICS OF THE DIAPHRAGM

PREPARATION (L)—Kill a rabbit or guinea-pig by bleeding Tie a ligature round the inferior vena cava and the gullet, and remove the abdominal viscera, so as to expose the under surface of the central tendon of the diaphragm With a fine camel-hair pencil, brush away the epithelium covering it, and then silver it in the usual way (p xlv) After exposure to light mount a small piece in dammar

EXAMINATION (L)—Observe the network of lymphatic vessels, some with a small, others with a large lumen Trace the course of a large vessel, and observe the bulgings in its course with narrower parts between (Pl XVII, Fig 1) Especially at the division of a vessel a valve may be seen (H) Study the character of the epithelium lining the vessels, and notice its sinuous outline

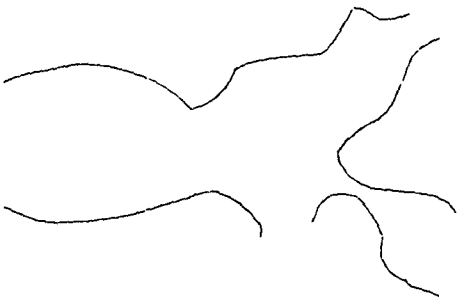
Lymphatics of the Intestine—For a large class, silvered lacteals are readily seen in the preparation of silvered muscular fibre obtained from the small intestine of a rabbit (p 37) Refer to that preparation, and observe details in the structure of these vessels similar to those described in the lymphatics of the diaphragm

The lymphatics communicate with the serous cavities by means of small apertures—*stomata*—guarded by small granular cells The stomata are most easily obtained from the septum of the great lymph-sacs of the frog, though they may also be obtained by silvering the under surface of the diaphragm of a rabbit Behind the stomach and on each side of the vertebral column of the frog there lies a large lymph-sac, separated only from the peritoneal cavity by a very delicate membrane or septum, which is perforated by small apertures—stomata—which bring the two cavities into direct communication

SEPTUM CYSTERNÆ LYMPHATICÆ MAGNÆ

PREPARATION—Kill a frog and carefully remove the abdominal viscera, taking care not to injure the fine septum above described Pour distilled water over the posterior abdominal wall immediately behind the stomach, when a delicate membrane will be floated up and brought into view Pour over it a half per cent solution of nitrate of silver till it

PLATE XVII LYMPHATICS



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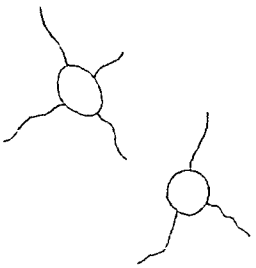

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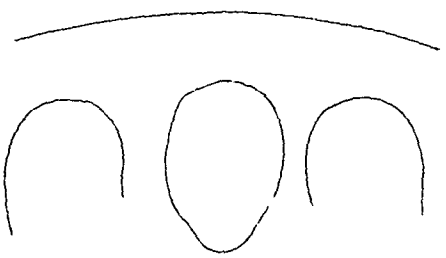

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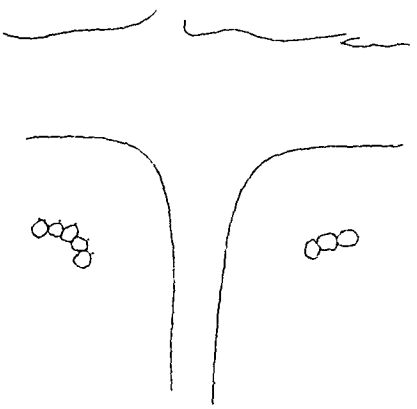

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becomes slightly white, snip out the membrane, and after washing expose it to light in the usual way Mount a small piece in glycerine

EXAMINATION (L and H)—Observe the membrane perforated here and there by small apertures—*stomata*—easily recognised by their having a brownish margin Their margins are bounded by small, plastic, granular cells, stained brownish with the silver (Pl XVII, Fig 2) The stomata may either be open or closed In addition, silver outlines are seen, and by focussing through the thickness of the membrane it is seen that epithelium exists on both surfaces, and that the size and shape of the cells differ on the two sides On the peritoneal surface the cells are elongated, while on the side directed towards the lymph-sac they are more or less polygonal and much broader Similar stomata exist on the mesogastrium and mesentery of the frog, and in the intercostal pleura, &c, of mammals They are prepared in the same way

LYMPHATIC GLANDS

(A) COMPOUND LYMPHATIC GLANDS

Lymphatic glands are accumulations of adenoid tissue (p 29) in the course of the lymphatic vessels, and so they have afferent and efferent vessels Each gland is surrounded by a connective-tissue capsule, which not unfrequently (ox) contains non-striped muscle, especially in the deeper layers From its under surface a number of trabeculæ carrying blood-vessels pass into the substance of the gland, thus dividing it into a number of compartments or alveoli, which are filled with a network of delicate adenoid tissue, whose meshes are in great part filled with lymph-corpuscles The gland-substance consists of a cortical and a medullary part In the cortex the adenoid tissue is arranged in the form of small, round, or oval nodules or *follicles*, and in the medulla as interlacing cords The lymph passes through well-marked channels in the gland—the *lymph-spaces*—which are spaces lying between the septa or trabeculæ and the gland-substance These spaces are lined by squamous epithelium, and across them is stretched adenoid tissue, which contains few lymph-corpuscles The structure of adenoid tissue has already been considered (p 29)

PREPARATION (*a*)—Place a lymphatic gland from an ox or other animal in Muller's fluid for a week, and then harden it in spirit and make sections (*b*) Harden a gland in a saturated watery solution of picric acid for twenty-four hours, and preserve in alcohol Stain a Muller's fluid section with logwood and mount it in dammar, and stain a picric acid section with picrocarmine and mount it in Farrant's solution

Lymphatic Gland (Logwood and dammar) **EXAMINATION** (L)—Observe the capsule with its septa subdividing the cortex into a number of compartments of *alveoli* As they pass into the centre of the gland the septa break up into *trabeculæ*, which run in all directions, and anastomose with each other, forming a plexus with relatively small meshes, so that it is easy to distinguish the small-meshed medulla from the cortex Observe the larger alveoli in the cortex Notice the masses of leucocytes stained deep blue, and between these and the trabeculæ narrow spaces, the lymph-sinuses (*Indicate the capsule, cortex, and medulla in* Pl XVII, Fig 3) (H) Observe the two layers of the capsule, the superficial layer contains connective tissue and a few elastic fibres, the deep one also non-striped muscular fibres Study one of the trabeculæ, which consist of connective tissue with a few elastic fibres, and observe the nuclei of the non-striped muscle The trabeculæ are cut in every direction, and branch

frequently Continuous on each side with the trabeculæ is the adenoid tissue, stretching across the lymph-space (Pl XVII, Fig 4) Blue-stained nuclei are seen at the nodal points of the fibres (Pl XVII, Fig 6) As already shown (p 29), they are the nuclei of squames which cover these fibres The meshes contain few leucocytes. Study a patch loaded with lymph-corpuscles, and also a transverse section of a trabecula with its lymph-space around it (Pl XVII, Fig 5) This is most easily found in the medulla

The **picric acid** and **picrocarmine** section shows similar details

Refer also to the preparations of adenoid tissue prepared by the *interstitial injection* of a solution of *silver nitrate*, which demonstrates the endothelium lining the lymph-sinuses (p 29)

Injection of the Lymph-Sinuses **PREPARATION**—With a hypodermic syringe inject into the lymphatic gland of an ox a two per cent watery solution of Prussian blue Place the gland in alcohol for twenty-four hours, and then make transverse sections Stain a section with carmine, and mount it in Farrant's solution

EXAMINATION (L)—Observe the distribution of the blue deposit *around the follicles* in the cortex, and around the trabeculæ in the medulla This method indicates beautifully the position of the lymph-sinuses

Blood-vessels—Study a section where the blood-vessels have been injected, and note that the large vessels lie in the trabeculæ, whilst the capillaries are almost entirely confined to the glandular substance

(B) SIMPLE LYMPHATIC GLANDS

These consist of masses of adenoid tissue occurring in the form of spherical or nodular masses, either singly or in groups, or sometimes in the form of cords They have already been referred to as occurring in the tonsils (p 65), at the root of the tongue (p 61), epiglottis (p 53), trachea (p 54), and lungs (p 59), (especially in the lungs of the cat), in the pyloric end of the stomach (p 67), throughout the intestine as solitary glands (p 70), and in groups, as Peyer's patches, in the small intestine (p 69) They also occur in the spleen (p 79) as Malpighian corpuscles They form the basis of the thymus gland (p 81)

THE SPLEEN

It is desirable to examine the spleen of one of the lower animals, say a cat, as well as that of man

(A) CAT

PREPARATION—Cut the spleen of a *cat* into five or six pieces, and harden them first in Muller's fluid, at the end of a week place them in chromic acid and spirit mixture, and after another week transfer them to spirit Make transverse sections Stain a section in logwood and mount it in dammar

(B) HUMAN

Cut a fresh normal human spleen into small pieces, and harden them in the same way as the above, but leave them two weeks in the chromic acid and spirit fluid Make sections of a part, and include the fibrous capsule Stain a section in logwood, and mount it in Farrant's solution

Transverse Section of a Cat's Spleen **EXAMINATION** (L)—Observe the serous covering and also the *fibrous capsule*, thick and firmly adherent to the subjacent organ Trace the large coarse fibrous *trabeculæ* passing from its under surface into the organ, where they anastomose and form a *trabecular framework*, which divides the organ into compartments of unequal size, that communicate with each other, and are filled with the splenic pulp The splenic pulp consists of a large number of cells like lymph-corpuscles, mixed with yellow blood-corpuscles In it observe the rounded aggregations of lymph-corpuscles constituting *Malpighian or splenic bodies* They are far more numerous relatively than in the human spleen, and lie scattered irregularly in the pulp They are not, as occasionally described, always spherical bodies, but in reality are cord-like masses of adenoid tissue, like those of the lung, developed on the walls of the arteries, usually more on one side of it than on the other, so that one must look for a section of an artery in each mass, and the position of the artery is eccentric Sometimes they are oval or spherical in shape In the trabeculæ search for sections of the large branches of the splenic artery and vein, which run for a certain distance together in these trabeculæ It is to be observed that the connective tissue which passes in on the blood-vessels at the hilum becomes continuous with the trabeculæ of the spleen itself There is no lymph-space between the trabeculæ and the pulp (*Indicate the general arrangement in one half of* Pl XVIII, Fig 1)

(H) Observe the *capsule*, consisting of several layers of fibrous tissue intermixed with a few elastic fibres and some non-striped muscle Select a *Malpighian body* Observe that it is made up of leucocytes, lying in a meshwork of adenoid tissue It has no definite wall, its outer boundary is indicated by the leucocytes being more crowded together, and so it is stained darker It is a mass of adenoid tissue developed in the adventitia of an artery, therefore look for a section of an artery in it, cut either transversely or longitudinally—The *Pulp* Observe the colourless corpuscles, and note the admixture of a large number of blood-corpuscles The fine network of delicate fibrils in which they lie imbedded may be observed

Human Spleen (Logwood and Farrant's solution) **EXAMINATION** (L)—Observe the capsule, trabeculæ, and splenic pulp In the latter notice a mottled yellow and bluish appearance The yellow streaks indicate the position of the blood-stream in the splenic pulp All the leucocytes are stained blue Observe the relatively small number of splenic corpuscles, which are usually ill-defined The splenic corpuscles can usually be best seen in the spleen of a young person, especially if the spleen be congested, small masses of yellowish-brown pigment may be found in the pulp

(H) A *Malpighian corpuscle* consists of a vascular mass of adenoid tissue, viz, a meshwork of adenoid reticulum loaded with leucocytes, many of which have two spherical nuclei (Pl XVIII, Fig 3) At their periphery they shade into the splenic pulp The *splenic pulp* is the most difficult part of the spleen to understand Select a thin fragment at the margin of the section, and observe the fine network of delicate fibrils crowded with leucocytes, and a very large number of coloured blood-corpuscles The latter give the pulp its yellow mottled appearance The framework or matrix of the pulp, when seen in sections, appears to consist of fine fibrils, which form a network with very small meshes, varying in size from a coloured blood-corpuscle to three or four times that size It is probable, however, that it consists of nucleated, branched, cell-plates, which anastomose so as to produce a honeycomb arrangement (Klein) They not unfrequently contain (especially in the congested human spleen) small masses of fine or coarse yellow pigment It is important to observe that this fine meshwork is directly continuous with the radicles of the veinlets The cell-plates arrange themselves into

the form of a continuous tube, and at the same time they become more spindle-shaped This is the mode of origin of a venous radicle from the pulp, so that the spaces in the splenic pulp are directly continuous with the vascular system (*Indicate these details in* Pl XVIII, Fig 2)

BLOOD-VESSELS OF THE SPLEEN

The splenic artery enters the organ (at the hilum) along with the vein, lymphatics, and nerves, all of which are imbedded in the connective tissue, which becomes continuous with the splenic trabeculæ It runs for a short distance in these trabeculæ, but it soon leaves the vein, and its branches divide suddenly into a large number of smaller branches (*penicilli*) The small branches soon become more or less completely ensheathed by a solid mass of adenoid tissue, and this tissue constitutes the so-called Malpighian or splenic corpuscles These are supplied by distinct capillaries, which form a uniform network with wide meshes Other arterial branches open directly into the meshwork, or 'honeycombed matrix,' of the splenic pulp, which, as above described, opens into the rootlets of the veins (venous sinuses), and these again into the larger venous trunks lying in the trabeculæ No capillaries are found in the pulp, they are confined to the Malpighian corpuscles At the periphery of these corpuscles the capillaries open directly into the spaces of the pulp, which are therefore the channels of communication between the terminations of the arteries and the origin of the venous radicles The blood-stream in the splenic pulp, therefore, corresponds to the lymph-stream in a lymphatic gland

PREPARATION **Injection of the Blood-vessels of the Spleen** (*a*) **Carmine Gelatine Mass**—It is well to use a constant-pressure apparatus, and to cease injecting whenever the mass ceases to flow into the spleen Inject from the splenic artery a thin carmine gelatine mass If the human spleen be taken, select the spleen of a child After the injection is completed harden the organ in Muller's fluid The spleen of a rat does very well, but in all cases the animal ought to be killed by bleeding In the case of animals it is sometimes advantageous to wash out the blood-corpuscles of the spleen with a stream of salt solution before using the gelatine mass

(*b*) **Silver Nitrate Injection**—Wash out the blood-vessels with distilled water, and then throw in a quarter per cent solution of silver nitrate Harden in alcohol Sections of this show the endothelium of the venous sinuses

Make sections of the above, stain them with logwood, and mount them in dammar

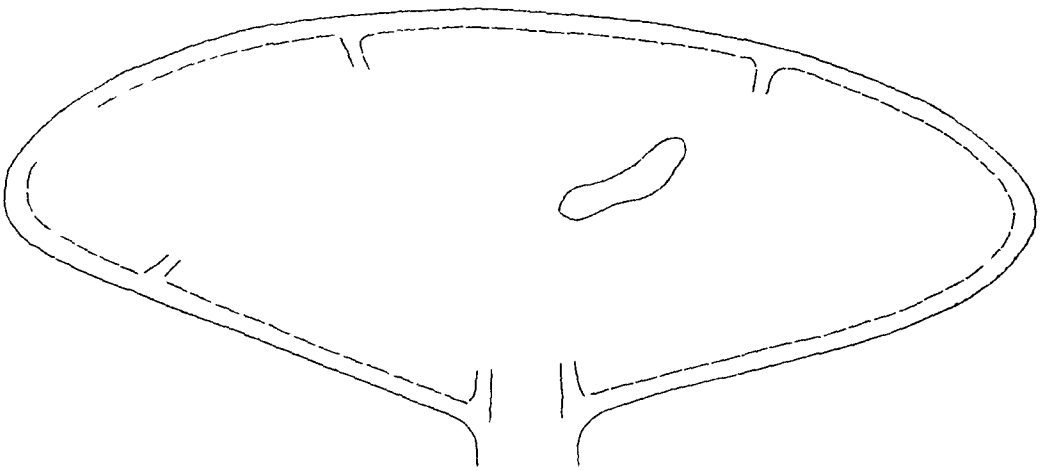
EXAMINATION (L)—Observe the Malpighian corpuscles, oval or irregularly shaped bodies stained blue, and in each note a branch of the splenic artery and a few capillaries The pulp contains a network of spaces (venous sinuses), filled with the red carmine mass, and in this lie the masses of logwood-coloured 'pulp' of the spleen, which are about the same breadth as the venous or cavernous sinuses Larger spaces—the rootlets of a vein—may be seen opening directly into these spaces (*Indicate the blood-vessels in one half of* Pl XVIII, Fig 1)

(H) Note the network of spaces filled with the red mass, and between them the lymph-cells stained blue Select a venous radicle, and note the layer of endothelium lining it Trace the continuation of an intra-Malpighian capillary into the red network at its margin

NERVES OF THE SPLEEN

These have already been alluded to (p 45) Amongst the non-medullated nerve-fibres, I have found a large number of 'plasma-cells' (p 20)

Plate XVIII Spleen & Thyroid Gland



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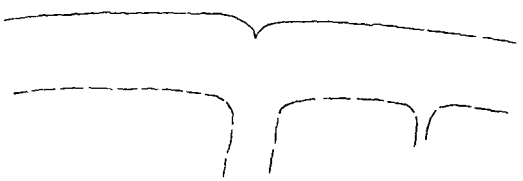

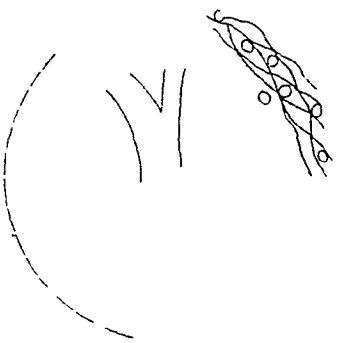

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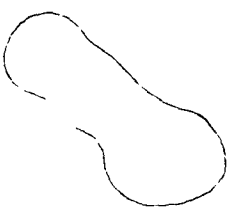

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FRESH SPLEEN

Cut across a fresh ox spleen Observe the capsule, trabeculæ, and dark grumous-like pulp The pulp can easily be washed away with a stream of water or salt-solution from a wash-bottle, the trabecular framework is then easily observed Observe the Malpighian corpuscles, small white bodies about the size of the head of a pin Do not mistake transverse sections of the trabeculæ for them Pick out one, and examine it in salt solution (H) It is seen to be made up of lymph-corpuscles, as already described

SPLENIC PULP

Tease a small part of fresh splenic pulp in salt solution, and apply a cover-glass

EXAMINATION (H)—Observe the lymph-corpuscles, perhaps the endothelial cells from a vein, also cells containing particles of fat, others with coloured blood-corpuscles, and some containing pigment, besides numerous coloured blood-corpuscles If the coloured blood-corpuscles are so numerous as to obscure the view of the other elements, tease the pulp in water, which dissolves the blood-corpuscles, thus the other elements can then be seen more distinctly

THE THYMUS

The thymus consists of an aggregation of lymph-follicles, or masses of adenoid tissue held together by connective tissue, which contains blood-vessels, lymphatics, and a few nerves The framework consists of a capsule of fibrous tissue, which gives off septa dividing the gland into *lobes*, these being further subdivided by finer septa into *lobules*, the lobules being subdivided by fine intra-lobular lamellæ of connective tissue into *follicles* The *gland-substance* is made up of these *follicles*, which are more or less *polygonal* in outline from mutual pressure Each follicle consists of a cortical and a medullary part, and the matrix or framework of each consists of a *fine adenoid reticulum* (p 29), made up of nucleated branched cells, which are easily seen In this meshwork the lymph-corpuscles lie In the medulla are found the so-called *concentric corpuscles* (Hassall) They consist of a central granular part, around which are disposed concentrically layers of flattened nucleated endothelial cells, and on section they present appearances like the 'cell-nests' that occur in epithelioma

PREPARATION (*a*)—Harden part of the thymus of an infant in chromic acid and spirit mixture for two weeks, and then transfer it to alcohol Stain a section with logwood and mount it in dammar

(*b*) Harden a similar piece in picric acid for twenty-four hours, and then transfer it to spirit Sections of this may be beautifully stained with picrocarmine Mount it in Farrant's solution The thymus of a kitten or puppy may be used, but these do not show the concentric corpuscles so well

EXAMINATION (L)—Observe the capsule, the interlobular and intralobular septa of connective tissue, made up of lamellæ with flattened corpuscles between them Note the polygonal or irregularly shaped follicles *Study a follicle* Observe the cortical part stained more deeply with the dye than the medulla (H) Search for the adenoid reticulum of

branched cells, and study the concentric corpuscles The reticulum is best seen in preparations mounted in Farrant's solution or glycerine

The concentric corpuscles can easily be isolated by teasing a small part of a fresh gland in salt solution

THE DUCTLESS GLANDS

These are the *thyroid gland*, *hypophysis cerebri*, the *coccygeal* and *carotid glands* of Luschka, and the *supra-renal body* They have no special ducts, and their secretions are probably removed by the lymphatics

THE THYROID GLAND

It consists of a capsule and a connective-tissue framework, which subdivides the glands into lobes and lobules Each lobule is made up of a larger or smaller number of gland-alveoli or *gland-vesicles*, which are closed sacs of various sizes, and are spherical, oval, or irregular in shape Each sac or alveolus consists of a membrana propria lined by a single layer of more or less columnar nucleated cells The size of the central lumen depends essentially on the amount of secretion These sacs are filled with a transparent, albuminous, viscid, slightly yellow-coloured fluid It is the accumulation of this fluid, and the consequent distension of these sacs, which constitutes goître Not unfrequently the alveoli contain large numbers of coloured blood-corpuscles (Baber) Each alveolus is surrounded by a rich plexus of capillaries which do not penetrate the membrana propria As in other glands, the large blood-vessels, lymphatics, and nerves lie in the interlobular connective tissue

PREPARATION —Harden the thyroid of a child in Muller's fluid for three weeks, or in chromic acid and spirit mixture for two weeks Then transfer it to spirit Make sections, stain them with logwood, and mount them in dammar

EXAMINATION (L)—Observe the interlobular connective tissue and the gland-vesicles, which are of different sizes and lined by a single layer of columnar epithelium (*H*) Study the membrana propria and epithelium of the gland-vesicles (Pl XVIII, Fig 4)

Blood-vessels of the Thyroid —They are easily injected when the whole body of a child or young animal is injected, but the blood-vessels are usually so filled naturally with blood that an injection need not be made Mount sections—stained or unstained—in dammar

THE SUPRA-RENAL CAPSULES

The *framework* consists of a fibrous tissue capsule, which sends trabeculæ into the interior of the organ, which is divided into a *cortex* and a *medulla* In the outermost zone of the cortex the trabeculæ form a plexus with polygonal meshes, whilst in the middle zone they are thinner and have a longitudinal arrangement, *i e* radiate inwards, in the innermost zone of the cortex they again form a polygonal meshwork A similar plexus of trabeculæ exists in the medulla These meshes are filled with the *parenchyma* or *gland substance*, which varies in appearance in the different zones In the outer cortical zone they are nucleated, granular, polyhedral cells, in the middle zone transparent nucleated cells, often containing oil-globules, and arranged in the form of cylinders, which anastomose with each other, whilst in the inner cortical zone the cells frequently contain a yellowish-brown pigment With these

are continuous the transparent, brittle, and sometimes pigmented and branched cells of the medulla Both cortex and medulla are richly supplied with blood-vessels, the distribution of which follows that of the trabeculæ A large number of nerves and nerve-ganglia exists in the organs

PREPARATION — It is best to employ the capsules of large mammals, *e g* of the ox, or, best of all, the horse If these cannot be obtained, use the supra-renals of a guinea-pig In the case of the human organ, it must be obtained as fresh as possible (*a*) Harden pieces in Muller's fluid for three weeks, or in chromic acid and spirit mixture for two weeks, and then transfer them to spirit Make transverse sections, and stain them with logwood, and mount them in dammar

(*b*) *Osmic Acid* —Place pieces about the size of a pea in a quarter per cent osmic acid for eight hours, and complete the hardening in spirit This method gives excellent results, but it must be remembered that the osmic acid is apt to fix only the outer layers

EXAMINATION (L) —Observe the capsule and its trabeculæ, also the cortex with its three zones and the medulla, and note the partially pigmented cells (Pl XX, Fig 4) (H) Study the cylinders of cells, and compare them with the foregoing description (Pl XX, Fig 5)

Study the injected specimen, and note the distribution of the blood-vessels

THE KIDNEYS

THE COURSE AND THE STRUCTURE OF THE URINARY TUBULES

THE course of the uriniferous tubules is very complicated, and the epithelium differs in different sections of the tubules

The urinary tubules begin as a blind extremity at the Malpighian corpuscle, and open on the free surface of a papilla Every tubule consists of the following sections, which are continuous with each other

(1) The *Malpighian capsule*, which passes by (2) a short, narrow neck or constriction into (3) the *proximal convoluted tube*, which passes into (4) the *spiral tubule* (Schachowa), lying in a pyramid of Ferrein All the above lie in the cortex As the spiral tube passes from the cortex into the boundary layer, it suddenly becomes straight and narrow as (5) the *descending limb of Henle's loop*, which is continued into the beginning of the papillary portion, where it bends on itself and forms (6) *Henle's loop*, which ascends into the boundary layer, where it suddenly enlarges and becomes slightly wavy, to form (7) the first *thick part of the ascending limb of Henle's loop* It again becomes narrow and spiral, when it forms (8) the *spiral part of the ascending limb* It now (9) re-enters the cortex, and, becoming narrower, ascends in a pyramid of Ferrein, which it soon leaves, and enters the labyrinth amongst the convoluted tubules, as (10) the *irregular tube*, so called because of its irregular size, breadth, and course This passes into (11) the *intercalated segment* (Schweigger-Seidel) which is the *distal convoluted tube*, exactly like (3) It passes into (12) the *curved part of the collecting tube*, a narrow curved tube which passes through the labyrinth, where it is joined by other similar tubes, and forms (13) the straight part of the *collecting tube*, which descends in a pyramid of Ferrein, and then enters the boundary portion, and finally passes into the papillary portion, there to be joined by other branches, and form a large (14) *collecting tube*, opening on the free surface of a papilla (Klein)

The membrana propria of Bowman's capsule is continuous throughout all the sections of the urinary tubules, but its epithelial lining varies greatly in different parts of its course

Bowman's capsule is lined with a single layer of squames , the proximal convoluted tubule (3) with a single layer of low nucleated columnar epithelial cells, which leave a lumen the size of one-third the diameter of the tube All the other sections of the tube, except the descending part of Henle's loop (5), and all the parts of the collecting tube (12, 13, and 14) are lined with polyhedral nucleated epithelial cells, whose outer half contains vertically placed 'rods' or fibrils (p 87)

The descending limb of Henle's loop (5 and 6) is lined with a layer of very flat transparent

nucleated plates, and therefore not unlike blood-vessels The ascending limb (7) in its wide part is lined with polyhedral 'rodded' cells, with their nuclei next the innermost part of the cells The narrow ascending part (8) is lined with low polyhedral cells, and has a very narrow lumen The collecting tubes are lined with a single layer of homogeneous transparent nucleated cells, which vary considerably in shape

It is to be remembered that the above arrangement has only been made out after laborious investigations, and no such complete tubule was ever seen, or can ever be seen, in a section of a kidney The course of the tubules can only be made out by isolating the tubules as described at p 86

PREPARATION *a* **Chromic Acid and Spirit Mixture**—Remove the kidneys from an animal just killed by bleeding—rabbit, dog, or cat, divide one transversely into several pieces with a sharp razor, and place them in the above fluid Take great care, in order not to separate the capsule Divide the other longitudinally and treat it in the same way Make both transverse and longitudinal sections of the kidney Stain some with logwood and mount either in dammar or in Farrant's solution, whilst others may be stained with picrocarmine and mounted in Farrant's solution

b **Muller's fluid** may be employed in the same way as the above, but takes from three to four weeks to complete the hardening

c **Ammonium Chromate** (Heidenhain) Harden small pieces in a five per cent solution of ammonium chromate for forty-eight hours, then wash away all the colouring matter, and complete the hardening in dilute, and then in strong spirit

Prepare portions of a normal *human* kidney in the same way (*a* and *c*) Compare sections of these with the above

KIDNEY OF A RABBIT OR DOG

Transverse Section **EXAMINATION**—With the *naked eye* note the general shape of the section, distinguishing the *capsule*, which is very easily detached from the cortex Note the division of the *parenchyma* into three distinct regions the *cortex*, the *boundary layer* (Ludwig) and the *papillary portion*, the two last forming the *medulla*, which terminates in a papilla, projecting into the pelvis of the kidney

(L) In the *medulla* observe the radiating rows of straight tubules (*tubuli recti*) proceeding from the pelvis outwards As they pass outwards they divide dichotomously When taken collectively they constitute a *Malpighian pyramid* In the *cortex* observe that on the boundary line between it and the medulla, the straight tubules of the Malpighian pyramid are continued as bundles of straight tubules into the cortex, constituting the *pyramids of Ferrein*, or *medullary rays*, which are the direct continuation of the straight tubes of the medulla into the cortex As they radiate towards the capsule these bundles of tubules become smaller, and are always separated from the capsule by a layer of convoluted tubules Observe the convoluted tubules (*tubuli contorti*) confined to the cortex They lie between the pyramids of Ferrein, and between their upper ends and the capsule Between two pyramids of Ferrein observe the double row of *Malpighian corpuscles*, which consist of the *capsule of Bowman* inclosing a *glomerulus*, or tuft of blood-vessels Sometimes the glomeruli fall out, and then there is only a circular aperture in the section Notice under the capsule more or less triangular spaces, which are transverse sections of veins Observe how easily the capsule separates, and note the very limited amount of connective tissue which passes from its under surface into the organ (*Indicate the shape of the kidney and the general arrangement of the tubules in* Pl XIX, Fig 1)

HUMAN KIDNEY

Examine (L) a similar section of a **human** kidney, and note especially the normal thickness of the capsule and its delicate and scanty fibres of attachment to the subjacent organ (*Indicate the capsule, pyramids of Ferrein, convoluted tubules, and Malpighian corpuscles in* Pl XIX, Fig 2)

(H) Select a **Malpighian corpuscle** Observe the membrane or capsule of Bowman, consisting of an elastic membrana propria, and note the single layer of squamous epithelium lining it The nuclei are stained, and bulge slightly into the cavity Outside it there is a small amount of fibrous tissue Within it note the tuft of blood-vessels, with their nuclei stained, and the capillaries, perhaps, containing yellow blood-corpuscles (Pl XIX, Fig 3) The capillaries are arranged in two or three conical lobules, but do not completely fill the capsule, the space between the surface of the glomerulus and the capsule depends on the amount and nature of the secretion present in it Between the capillaries there is a very small amount of connective tissue (chiefly branched connective-tissue cells), which holds them together, and over them there is a layer of squames The section may pass through the capsule in the plane of the afferent or efferent blood-vessels, or the narrow neck which connects the cavity of the capsule with a convoluted tubule may be found

Select a convoluted tubule (H)—Observe them, one divided longitudinally and another transversely, and observe the limiting membrane, and how closely they are packed in the cortex, with very little supporting tissue between them Study the secretory epithelium lining them, and note that the lumen of the tube is small, that the epithelial cells are ill-defined and have no cell-wall They contain a spherical nucleus placed near the attached end of the cell, and their protoplasm is always cloudy and granular The outer part of the protoplasm is often striated, this is especially the case if the section has been hardened in ammonium chromate Some tubules may be found with an angular zigzag bend in them, *these are the connecting tubules* (*Indicate a convoluted tubule in* Pl XX, Fig 1)

Select a Pyramid of Ferrein (H)—Observe the straight tubules, and amongst these the *looped tubules of Henle*, with their descending limb, which is very narrow and lined by flattened epithelium, with alternately projecting nuclei on either side They are not unlike capillary blood-vessels, though they have a well-marked basement-membrane *It is difficult to distinguish* the ascending limb, as it is so like a collecting tubule The descending branch may be traced downwards into the medulla (*Indicate these tubules in* Pl XX, Fig 1)

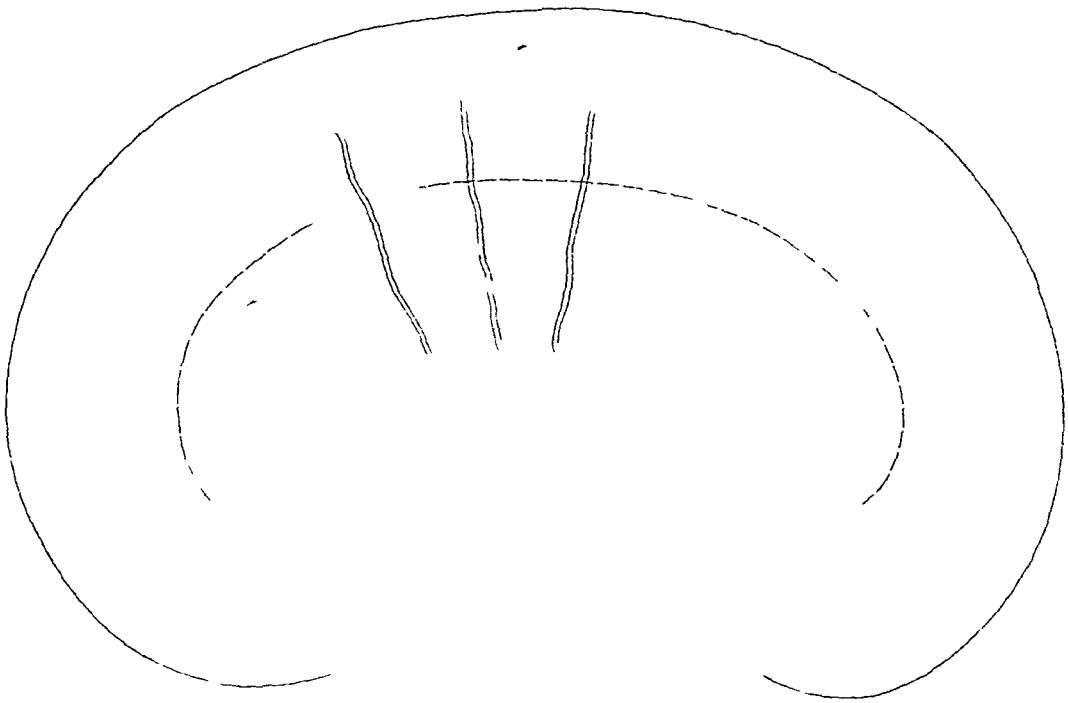
The Medulla (H)—Observe the straight tubules—*collecting* or *discharging tubes*—which open on the apex of the Malpighian pyramid Observe their columnar or cubical epithelial covering The cells are well-defined, and contain a well-marked spherical nucleus, imbedded in clear transparent protoplasm There is a well-marked lumen in the centre of the tubule Observe the basement-membrane in which the cells are placed, and note the relatively large amount of connective tissue in the medulla compared with the cortex (Pl XX, Fig 2)

Place unstained sections of a kidney in a quarter per cent osmic acid for twenty-four hours, and mount them in Farrant's solution

ISOLATED RENAL TUBULES

PREPARATION—The arrangement and relation of these parts one to another cannot be made out in a section, to do so the kidney must be boiled for a long time in a mixture of alcohol and hydrochloric acid, which dissolves the connective tissue and liberates the tubules A very good method is to place pieces of a kidney, about the size of a pea, in

PLATE XIX. KIDNEY



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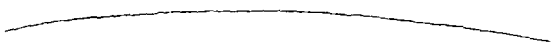

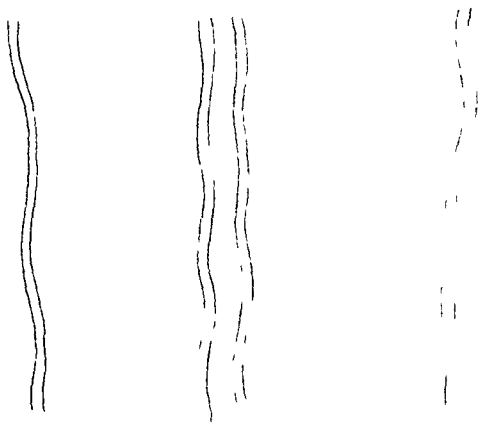

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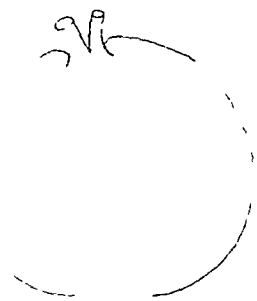

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pure hydrochloric acid for twelve hours, and then leave them in water for twenty-four hours Tease out a small piece in osmic acid solution (one per cent), and mount it in Farrant's solution By teasing part of a kidney prepared in this way it has been shown that the Malpighian corpuscle is continuous with a convoluted secreting tubule, which winds about in the cortex, and then descends in a pyramid of Ferrein into the medulla, forming the descending part of the looped tubule of Henle, and reascends as the ascending part of the loop, and after a short course as a connecting tube, with a zigzag direction, becomes continuous with a collecting tube, and descends through a pyramid of Ferrein, and in its course joins at an acute angle with similar tubules, which is thus a discharging tube, opening on the surface of a papilla

CONNECTIVE TISSUE OF THE KIDNEY

Compare this section with the above Make transverse sections through the cortex, and also through the medulla near the apex of a Malpighian pyramid, and stain them with logwood or picrocarmine, and mount in Farrant's solution

EXAMINATION (L)—**In the cortex** Observe the convoluted tubules and glomeruli, notice the small amount of interlobular connective tissue, and search for a transverse section of a group of straight tubules, *i e* of a pyramid of Ferrein Study the characters of these parts (H)

(L) **Apex of a Malpighian Pyramid**—Observe the transverse sections of the discharging tubules lined with clear, low, columnar epithelium, and a well-defined lumen Notice the large amount of connective tissue between and supporting the tubules, forming a marked contrast to the small amount in the cortex

It is desirable to have a transverse section through the boundary line between the cortex and medulla

Observe the transverse sections of the collecting tubules, and here and there the cut ends of the ascending and descending looped tubules of Henle, the narrow loop is not unlike a capillary with its bulging oval nucleus Sections of capillaries may also be found, but they usually contain blood-corpuscles Note the moderate amount of intertubular connective tissue—intermediate in amount between that of the cortex and medulla

Ammonium Chromate Kidney—Heidenhain showed that the epithelial cells lining a uriniferous tubule—excepting those of the descending limb of Henle's loop—the loop itself, and all parts of the collecting tube, are made up of a number of 'rods' or fibrils placed vertically to the long axis of the tube These rods are most distinct in the *outer* half of the cells, *i e* next the membrana propria Each cell contains a spherical nucleus placed about the middle of its substance

EXAMINATION (L)—Select a convoluted tubule in the cortex, and use (H) to see the above-described characters They are easily seen in a properly prepared kidney

BLOOD-VESSELS OF THE KIDNEY

PREPARATION—Make transverse and longitudinal sections of a kidney which has been injected from the renal artery with a gelatine and carmine or Berlin-blue mass Mount in dammar

EXAMINATION (L)—Observe large branches of the renal artery running outwards between the Malpighian pyramids, from these the *interlobular arteries* proceed vertically

outwards between the pyramids of Ferrein As the interlobular arteries proceed outwards, they give off on all sides small, short trunks—the *afferent arteries*—to the Malpighian corpuscles, which they enter and split up into capillaries to form the *glomerulus* (p 85) Trace an *efferent vessel—venous vessel*—emerging from the capsule at that part where the afferent vessel enters it The efferent vessel on its exit splits up into capillaries, which form a dense network over and around all the convoluted tubules From this network the renal vein proceeds Branches of it will be found accompanying the interlobular arteries Note at the innermost part of the cortex bunches of vessels, most of which spring from the renal artery and run down in groups into the medulla—the *vasa recta* - where they split up into a network with large oblong meshes surrounding the straight tubules The medulla is not nearly so vascular as the cortex, but the capillaries of the one are continuous with those of the other (Pl XX, Fig 3)

FRESH KIDNEY

Make a longitudinal section of a perfectly fresh sheep's kidney With the naked eye observe the capsule and its loose attachment to the parenchyma Note the division of this latter into three zones (p 85)—the *cortex*, *boundary*, and *papillary portion* Study the naked-eye characters of each part The *papillary portion* is uniformly vertically striated, due to the straight tubules and blood-vessels running straight towards the apex of the pyramid The *boundary-layer* is also striated longitudinally, but is marked by alternate opaque and light bluish-coloured zones, the former are the continuations of the straight tubules towards the cortex, whilst the latter are due to the vasa recta (p 88) The cortex is of a light brown colour, and is finely granular in appearance

It is important in a human kidney to note the *relative* thickness of these various parts If the vertical diameter of the cortex and medulla together be represented by 10, the relative proportions of these three zones from without inwards will be 3 5 2 5 4 (Klein) The medulla, therefore, is nearly twice as thick as the cortex

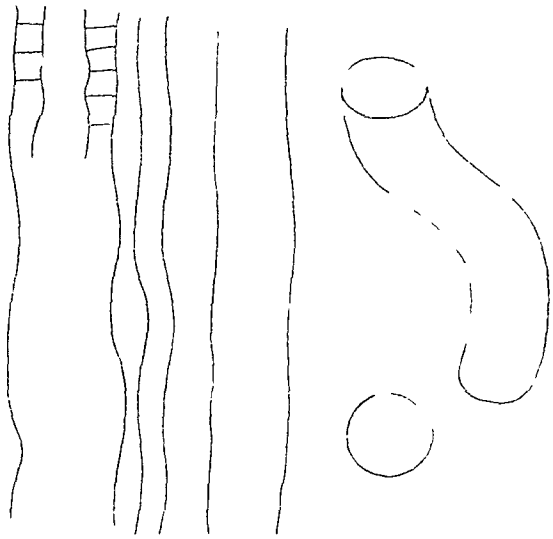
EXAMINATION (L)—Scrape off a small piece of the *cortex*, and tease it in salt solution with needles Observe the convoluted tubules with their cloudy epithelium, their nuclei being scarcely visible Notice especially their basement-membranes, which may be seen in great numbers in the field as fine transparent membranes, often with folds in them Study them also with (H) Select a glomerulus and study it Add dilute acetic acid, and observe the effect on the epithelium of the convoluted tubule, in rendering it clear and transparent and revealing a nucleus, and also its effect on the glomerulus in rendering its capillaries clear and transparent, and bringing into view their nuclei

Scrape also the *medulla*, and examine it in the same way (L and H) Note the straight tubules with their well-defined, clear, epithelial cells Perhaps isolated portions of the looped tubules of Henle may be obtained

THE URETER

PREPARATION—Tie one end of the ureter of a dog or cat, and slightly distend it with the chromic acid and spirit mixture, then tie the other end, leave it for two days in the mixture, then cut it into pieces an inch long, and harden it for two days longer, and complete the hardening in spirit Make transverse sections, and stain some with logwood, and mount them in dammar

PLATE XX KIDNEY & SUPRA-RENAL CAPSULE



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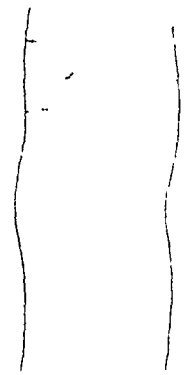

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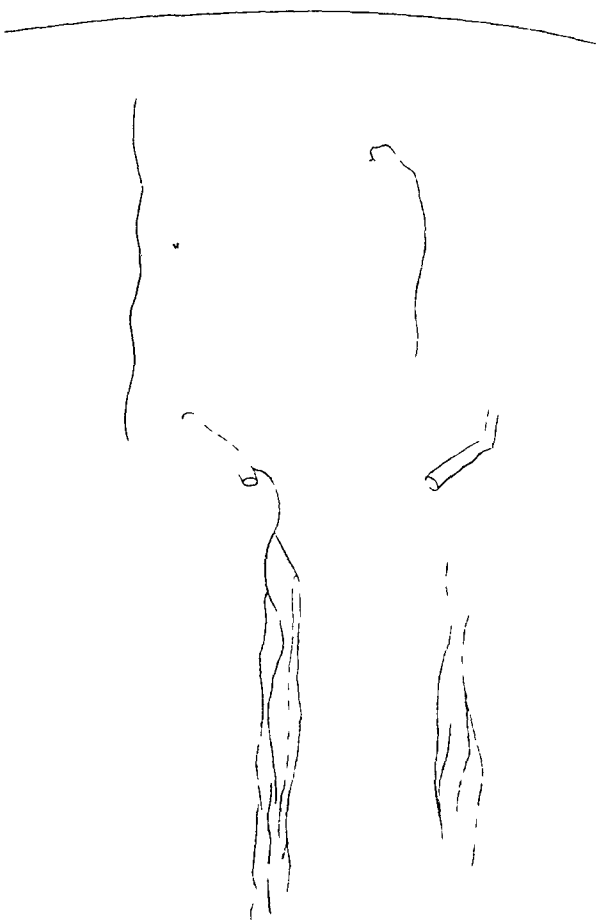

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EXAMINATION (L)—Note the (1) *external fibrous coat* of connective tissue, the (2) *middle* or *muscular coat*, consisting of non-striped muscle arranged in two layers—(*a*) an external circular, and (*b*) an inner longitudinal, with the muscle-cells composing it cut transversely, and (3) the *internal* or *mucous coat* lined by stratified transitional epithelium (H) Study each of these coats, but note the transitional epithelium (p 12), the upper cells are polyhedral, and below them is a layer of pear-shaped, or club-shaped, cells

THE BLADDER

PREPARATION—Prepare this exactly in the same way as the ureter Use the bladder of a cat or dog It must be cut into several pieces after being distended for two days Make vertical sections, and stain them with logwood, and mount in Farrant's solution

It has practically the same structure as the ureter Outside is the (1) *serous* coat, (2) the *muscular* coat, with its fibres arranged in three directions, (3) the submucous coat, (4) the mucous coat lined with stratified transitional epithelium

The **bladder of the frog** has already been alluded to (p 36)

The **nerve-ganglia** of the bladder and ureters are best studied by the lemon-juice and gold method (p xlv)

THE SKIN.

THE skin consists of (*a*) the *epidermis*, (*b*) the *corium* (cutis vera or true skin, with the papillæ), whilst deeper down is (*c*) *subcutaneous tissue* containing fat-cells

The **epidermis** is made up of several layers, the most superficial is (1) the *stratum corneum*, or horny layer, composed of layers of flattened epithelium about to be thrown off It varies in thickness, being thickest where there is most pressure, *e.g.* palms of the hands and soles of the feet Below this is (2) the *stratum lucidum*, a narrow, clear, homogeneous, easily-recognised layer In it the epithelial cells are closely packed together, and each one contains a flattened nucleus Then follows (3) the *stratum granulosum* (Unna), or the 'stratum of granular cells' (of Langerhans) It is best seen where the skin is thick, and consists of two or more layers of spindle-shaped 'granular' and nucleated cells, which become deeply stained with the carmine of picrocarmine, and also with logwood, so that it forms a marked feature in a stained section The 'granular' appearance is due to the presence of granules of some albuminoid matter, probably closely related to keratin The deepest layer (4), rete mucosum, or rete Malpighii, or *stratum Malpighii*, consists of several layers of epithelium, the deepest layers are composed of small columnar cells, with oval nuclei, whilst those of the middle layers are more or less polyhedral, with spherical nuclei In this layer is deposited the pigment in coloured individuals In it 'prickle' cells are easily seen This layer rests on the true skin, and also dips down between the papillæ of the true skin

The **corium** has, projecting from its superficial surface, a series of conical or cylindrical *papillæ*, which vary in size and number in different localities, being most numerous where the sense of touch is most acute, *e.g.* palms of the hands and soles of the feet The papillæ and the remainder of the true skin consist of bundles of fibrous tissue, which interlace in a very complex manner, so as to form a dense tissue, and in it are to be seen connective-tissue corpuscles and interfascicular lymph-spaces, and in it lie gland-ducts, blood-vessels, nerves, hairs, &c It also contains many elastic fibres, which are most numerous in the subcutaneous tissue and fewest in the papillæ They are arranged with reference to the surface of the trabeculæ of fibrous tissue

The **subcutaneous tissue** contains groups of fat-cells, and between them septa of fibrous tissue

The skin also contains sweat-glands, and in certain places sebaceous glands and hair-follicles, and numerous nerves and their terminations, blood-vessels, and lymphatics

PREPARATION (*a*) *Chromic acid and Spirit Mixture*—Place small pieces of the skin from various parts, *e.g.* from the palm of the hand, fingers, or sole of the foot, and scalp, in the above fluid for ten days, and complete the hardening in spirit Make vertical sections with a freezing microtome and stain some with logwood, and mount them in dammar, others should be stained with picrocarmine, and mounted in Farrant's solution, whilst others again are to be

doubly-stained, one set with picrocarmine and logwood (dammar), and another with picrocarmine and iodine-green (dammar), p xlvi

(*b*) Minute portions of skin may be hardened in a quarter per cent solution of *osmic acid* for twenty-four hours, and then preserved in spirit

(*c*) Prepare pieces of the skin of a human fœtus in the same way as (*a*), but keep them only one week in the hardening fluid

(*d*) Place portions of fœtal skin in *ordinary alcohol*

(*e*) *Artificial digestion*, p xxxiv

SKIN OF THE PALM

Vertical Section (Picrocarmine) **EXAMINATION** (L)—Observe the *epidermis*, consisting of many layers of stratified epithelium Beneath this the *corium*, stained bright red, with papillæ projecting into the epidermis, which dips down between the papillæ In the deeper part of the skin note the distinct passage of the connective tissue into that of the subcutaneous tissue, which latter often contains masses of fat Perhaps the duct of a sweat-gland may be seen running vertically in the corium, and terminating upwards in a passage which winds its way in a corkscrew-like manner through the epidermis In the subcutaneous tissue will be found sections of the gland, where it is arranged in the form of a coil Study these parts (*Indicate the general arrangement in one half of* Pl XXI, Fig 1)

(H) **Epidermis**—Observe the superficial squames, stained yellow, occurring in many layers (*stratum corneum*) They do not appear to contain a nucleus, are flattened, and seen on edge Beneath this note a clear layer, which does not stain well, and in which the outline of the cells is difficult to make out—the *stratum lucidum* Immediately below this there lies a layer of granular cells two or three deep, which are deeply stained with carmine, and so stand out brightly—the *stratum granulosum* (Langerhans) Below this observe the layers of more or less polyhedral cells, with their nuclei stained red Their edges often show prickles This layer, with those cells that lie below it, constitutes the *rete* or *stratum Malpighii* The layer of cells resting directly on the corium is composed of small columnar cells with oval nuclei They seem to be devoid of a membrane Compare the section of the lip (p 60) for pigment in these cells (*Indicate these layers of epithelium in* Pl XXI, Fig 2 *Their relative position is indicated by letters*)

These details, and especially the staining of the nuclei with the carmine, are better seen after the preparation has been kept for a week The nuclei gradually absorb the surplus dye from the Farrant's solution

Corium (H)—Observe the papillæ and the rest of the true skin, all stained of a deep red colour Note the red-stained bundles of connective tissue running and interlacing in every direction, and amongst them notice the large number of elastic fibres (stained yellow) arranged in the form of a network outside these bundles Trace the continuity of the connective-tissue bundles of the corium with those of the subcutaneous tissue Here and there in the papillæ capillary loops of blood-vessels, and perhaps nerves, may be seen (*Fill in papillæ in* Pl XXI, Fig 2)

Sweat-gland (H)—Observe the gland-coil lying in the subcutaneous tissue Note the gland-tube, cut in all directions Each tube consists of a basement-membrane, lined by low columnar or cubical cells Trace a gland-tube upwards through the corium It consists of a basement-membrane, lined with low columnar epithelial cells, which are continuous with, and are an extension of, the cells of the stratum Malpighii In all cases the gland passes upwards

between two papillæ From the epidermis it pursues a spiral course Each sweat-gland consists of a simple tube, composed of a coiled portion—the gland proper—situated in the subcutaneous tissue, and a duct, which passes more or less vertically through the corium, to open on the surface of the epidermis A brightly refracting membrane seems to line the duct in its passage through the epidermis In the corium, the *duct* consists of a membrana propria, and inside this are two or three layers of polyhedral nucleated cells, which are separated from the comparatively narrow lumen by a homogeneous bright membrane In the coiled part, the tube (proximal) is lined with similar cells, but in the other, or distal part, the membrana propria is very thin, and it is lined with a single layer of columnar nucleated cells, which are striated longitudinally (*Indicate a section of sweat-glands in* Pl XXI, Fig 3)

Touch-corpuscles (Wagner) may be found in some of the papillæ They are oval bodies, made up of fibrous tissue, and form one of the 'end organs,' or structures in which the nerves of the skin terminate

In the subcutaneous layers sections of *Pacinian corpuscles* may be found (p 46), but they will be more easily found in the fœtal skin (*Indicate a section of one in* Pl XXI, Figs 1 and 3)

DOUBLE-STAINING OF THE SKIN

This is a most excellent method for revealing the different structures

1 *Picrocarmine and Logwood*—Make vertical sections of the skin of the heel of a fœtus, and stain it first with picrocarmine Wash the section in water slightly acidulated with acetic acid, and then wash it thoroughly in ordinary water Stain it slightly in logwood and mount in dammar

The superficial layers of the epidermis are yellow, the deep layers and sweat-glands are a logwood tint, and the connective tissue is red

2 *Picrocarmine and Iodine-green*—Proceed as before, but use a very dilute solution of iodine-green (p xliv) instead of the logwood Mount in dammar

The superficial layers of the epidermis are yellow, the deep layers, the sweat-glands, nuclei of the fat-cells and connective-tissue corpuscles are green, whilst all the connective tissue is red These doubly and trebly stained sections are very instructive In these sections one is almost sure to meet with numerous sections of Pacinian corpuscles (p 46) Note also the relatively larger number of sweat-glands in the fœtal skin (compare p 27)

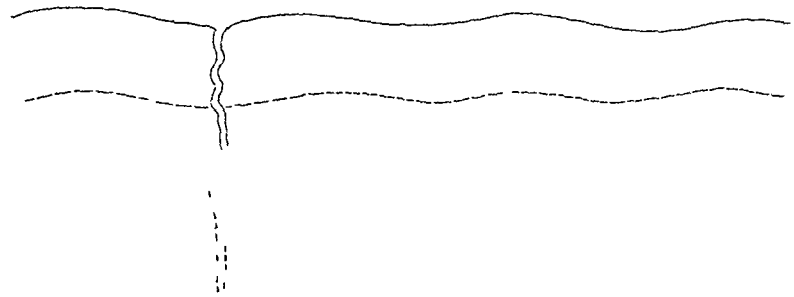
For the development of fat-cells see p 27

DIGESTION OF THE SKIN

This method was introduced into histology several years ago by the author It is invaluable for ascertaining the arrangement of the elastic fibres and muscular tissue in the skin It has recently been adopted by Kuhne for investigating the structure of nerves It depends for its value on the fact that certain substances are digested more rapidly than others, and so are rapidly removed

Make an *artificial gastric juice* by mixing 1 c c of pure hydrochloric acid with 500 c c water, and add one gramme of pepsine, or a few drops of a glycerine extract of the gastric mucous membrane It is well to keep the mixture at 38° C for two or three hours before using it The piece of skin to be digested is stretched over a small glass ring and firmly tied

PLATE XXI SKIN



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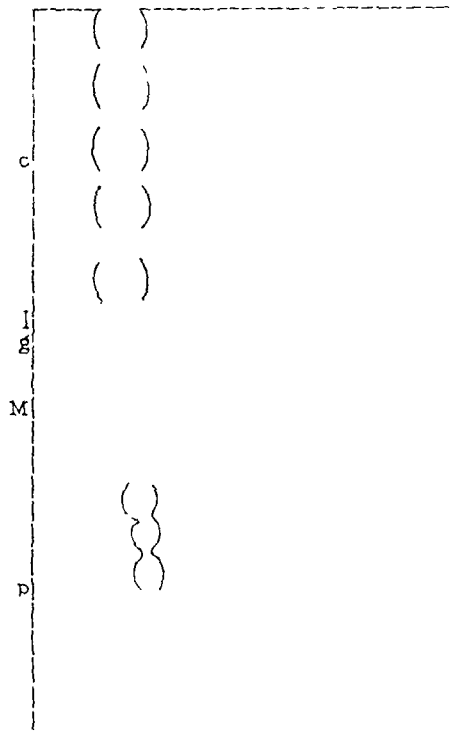

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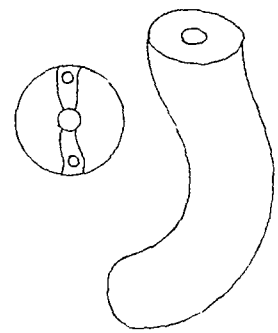

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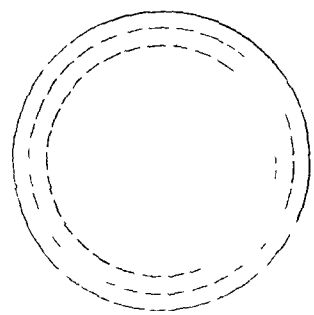

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to it It is then placed in some (200 c c) of the digesting fluid, which is kept at a temperature of 38° C in an ordinary water bath for a period varying from three to eight hours,—the time depending upon the age and size of the piece of skin After partial digestion the skin is placed in water for twelve hours, when it swells up and becomes extremely transparent It may be kept most advantageously in a ten per cent solution of common salt, or it may be hardened in one of the ordinary hardening fluids, and afterwards stained with logwood or carmine

This method is also applicable to other tissues (W Stirling)

HUMAN SCALP

Vertical Section **EXAMINATION** (L)—Make the sections parallel with the hair-follicles Stain a section with picrocarmine or logwood, and mount it in dammar Observe the epidermis and corium, and in the latter the *hair-follicles*, each of which gives origin to a hair Note its mouth, and its bulbous lower extremity, into which projects a vascular papilla The follicles are usually placed obliquely, and in the scalp they lie in groups of three or four, as is easily seen when a horizontal section is made Into the upper part or neck of the follicle one or two *sebaceous* glands open Rising a short distance above the bulbous portion is the *arrector pili*, composed of several bundles of non-striped muscle-cells It runs obliquely upwards through the corium, forming an acute angle with the follicle, and passes over the base of the sebaceous gland, and terminates in a plexus on the surface of the corium (*Indicate a hair-follicle and its muscle in* Pl XXII , Fig 1)

Study a Hair-Follicle (L and H)—Proceeding from without inwards, observe the (1) *outer* and (2) *inner sheath* of the hair-sac, which are continuous with the papillary layer of the corium In the outer layer the fibres are arranged longitudinally, whilst in the inner one they are transverse or oblique Both layers are best marked at the lower bulbous portion of the follicle, where they are pushed into the lower end of the hair, to form a club-shaped *papilla* Internal to these two layers is (3) a *glassy hyaline membrane*, which separates them from the epidermic coverings of the hair Inside this is (4) the *outer root-sheath*, which is thickest about the middle of the follicle, and consists of several layers of cells continuous with the stratum Malpighii Inside this is (5) the *inner root-sheath*, which in longitudinal sections appears as a thick glassy membrane, though it can be proved to consist of three layers, being from without inwards—(*a*) *Henle's layer*, then (*b*) *Huxley's layer*, and (*c*) the *cuticle of the root-sheath*, which is a very delicate membrane composed of imbricated non-nucleated scales The inner root-sheath terminates abruptly at the neck of the follicle Then follows (6) the *cuticle of the hair* and (7) the *hair* itself

Sebaceous Gland (L and H)—Observe the duct of one or more of these glands opening into the upper part of a hair-follicle The membrana propria (of the duct) is continuous with the glassy membrane (3) of the hair-sac, and the epithelium of the outer root-sheath is directly continuous with the epithelium of the duct The duct after branching opens into flask-shaped or saccular alveoli In these alveoli are found one or more layers of cells loaded with fat-globules (Pl XXII , Fig 2)

Arrector pili Muscle (L and H)—Observe its attachment to the lower part of the hair-follicle and trace it obliquely upwards towards the surface of the corium (p 91) It is composed of non-striped muscle

Horizontal Sections of the Scalp—Stain it with logwood or picrocarmine, and mount it in dammar Observe the transverse sections of the hairs arranged in groups of three or four

Make out the different layers of the hair-follicle Compare also the section of a lip of a dog (p 60) where these various coverings are exquisitely shown in sections of the large 'feelers'

HAIR

Human Hair—Pluck a hair from the head, and place its shaft in water Observe (H) its *cuticle*, consisting of imbricated scales, then the *cortex*, which forms the mass of the hair and is composed of horny matter made up of scales, which in pigmented hairs have fine granules of pigment deposited between them In the centre note the *medulla*, or *pith*, which is absent in some hairs (Pl XXII, Figs 3 and 4) A hair may be preserved dry, or simply by adding a drop of dammar solution and then applying a cover-glass, but after the addition of dammar the cuticle is not well seen

Hair of Rabbit—Treat it similarly, but note the single or double row of oblong compartments in the medulla filled with air, and therefore appearing black

Transverse sections of hairs are obtained by shaving, but the hairs of the beard are usually flattened ovals, whilst those of the head are round

NAILS

Make vertical sections of a nail and its nail-bed after hardening in alcohol or chromic acid and spirit mixture They are only peculiar modifications of the stratum Malpighii They rest on very vascular papillæ (bed or matrix)

By steeping them in forty per cent caustic potash solution, the nails can be resolved into their constituent cells (p xxxiv)

BLOOD-VESSELS OF THE SKIN

PREPARATION—It is best to employ the skin of the extremities, for a limb is easily injected from its main artery Inject a limb with a carmine-gelatine or Berlin-blue mass Harden parts of the skin in Muller's fluid for two weeks, and then in alcohol Make moderately thick vertical sections Stain the sections injected with a red mass with logwood, and the others with picrocarmine Mount them in dammar It is well to prepare similar sections of an injected scalp, to see the arrangement of the blood-vessels round a hair-follicle

EXAMINATION (L and H)—Passing from the subcutaneous tissue upwards we find the following systems (Tomsa) —

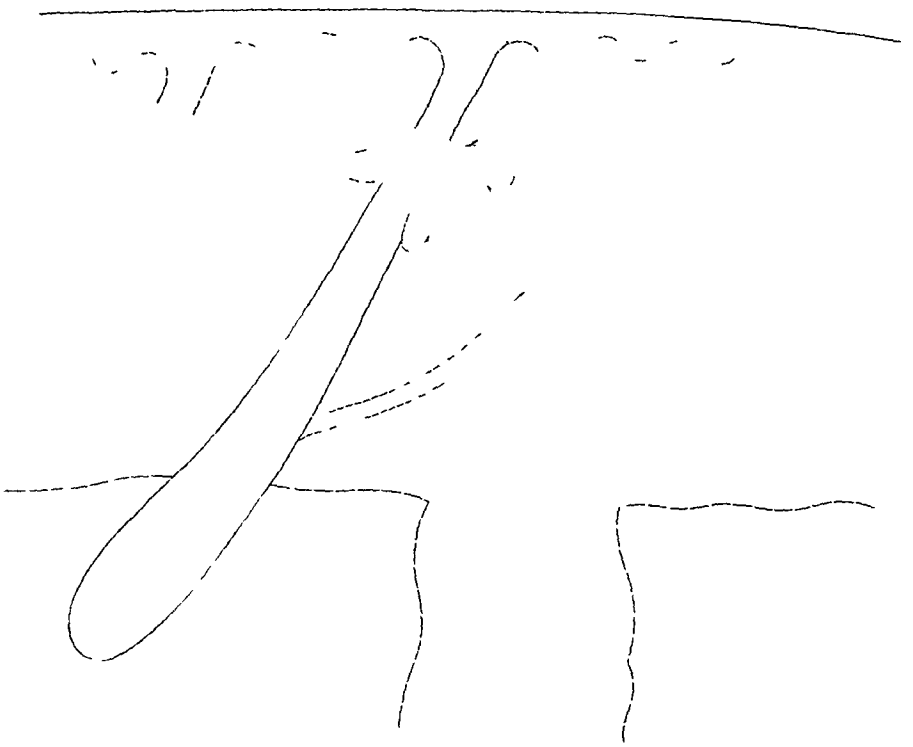
(1) Observe the fat-lobules, each supplied by a small artery which forms a dense plexus over and between the individual fat-cells, and from it emerge one or two veins

(2) A branch proceeds upwards to the coil of a sweat-gland, where it forms a plexus, whilst the duct is supplied by a branch from an artery in the corium where the capillaries anastomose

(3) Each papilla of a hair-follicle receives an arteriole, which splits up into capillaries within it A plexus of capillaries exists between the outer longitudinal and the inner circular coats of the hair-sac

(4) A capillary plexus exists round the sebaceous gland and arrector pili

PLATE XXII SKIN & HAIR



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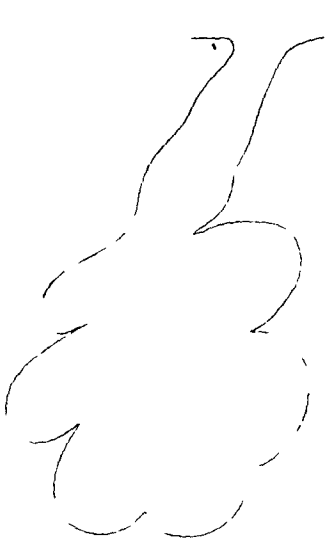

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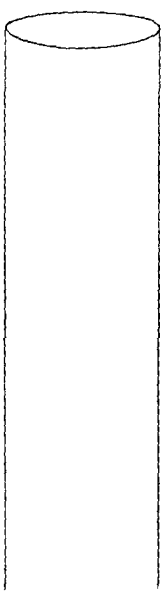

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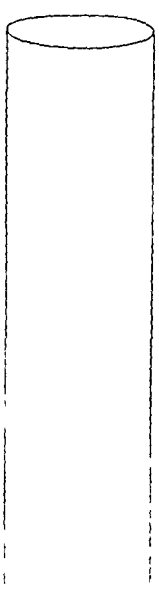

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(5) Trace an artery arising from a subcutaneous trunk, and note that as it ascends it gives off branches to the sweat-duct, hair-sac, sebaceous glands, and hair-muscle, and note its termination in capillary loops in the papillæ, from which a vein proceeds which opens into relatively large veins lying in the superficial layers of the corium (Klein) (*Indicate the general arrangement of the blood-vessels in* Pl XXI, Fig 1)

LYMPHATICS OF THE SKIN

They require to be injected by the puncture method (p lv), using a watery solution of Berlin-blue But the task is by no means an easy one Injection of the skin of the palm of the hand or sole of the foot yields the best results

NERVES OF THE SKIN

The nerves of the skin are very numerous, and the mode of termination of some of them in Pacinian corpuscles in the subcutaneous tissue (p 46), and in Wagner's tactile corpuscles in some of the papillæ of the corium (p 92), has already been alluded to These touch-corpuscles are most numerous in the lips, skin of the hand, foot, and glans penis

It requires some modification of the gold method—preferably that with lime-juice and formic acid (p xlv)—to obtain preparations of the various nerve-plexuses (sub-epithelial and inter-epithelial) in the skin

The snout of the pig and the skin of the nose of the mole may be employed

The peculiar nerve-endings recently described by Ranvier and Merkel, may be found in the snout of the pig, and especially in the soft part of a duck's bill

THE NERVOUS SYSTEM

THE SPINAL CORD

PREPARATION (*a*) **Muller's Fluid and Spirit Mixture**—Remove the spinal cord and medulla oblongata, with the pia mater attached, from a newly-killed cat, rabbit, or dog Indeed, all the membranes may be left attached to the cord Take care to avoid squeezing or stretching the cord It is not necessary to make any incisions into the cord at first Suspend it in a wide, cylindrical vessel—such vessels as are used for water analysis do very well—filled with a mixture composed of three parts of Muller's fluid, and one of methylated spirit The mixture ought to be made at the time, but as a considerable amount of heat is evolved when the fluids are mixed, it ought to be allowed to cool *Keep the tissues in a cool*—in fact, a *cold place* This is of great importance Renew the fluid at the end of twenty-four hours, and then again at the end of a week The cord may now be cut, with a sharp razor, into segments an inch or so long The cord ought to be hardened in this mixture for three weeks It is then transferred to a two per cent solution of ammonium bichromate for two weeks, to complete the hardening Preserve them either in spirit, or in a solution of chloral hydrate, of twelve grains to an ounce of water (Hamilton) A portion of the spinal cord of a man or an ox ought to be hardened in the same way

(*b*) **Potassic-bichromate**—Cut the fresh spinal cord of an ox into pieces an inch long by means of a sharp razor Place them in a large quantity of two per cent solution of potassic bichromate Do not let one piece lie on another See that they are separated by cotton wool, previously dipped in alcohol, to avoid pressure It requires from three to five weeks to complete the hardening Preserve the cord in spirit A cord hardened in this way shows the nerve-structures very well

(*c*) **Chromic Acid and Spirit Mixture**—The cord must be cut into pieces half an inch long and placed in this fluid, which will harden them in from two to three weeks It is not so well suited for showing the nerve-elements as for demonstrating the neuroglia

(*d*) **Spirit and Iodine, and then Ammonium-bichromate**—Suspend the cord in a long, cylindrical vessel, in methylated spirit tinged yellow with tincture of iodine Leave it in this mixture—usually three or four days—until the yellow colour has nearly disappeared Cut it in pieces an inch long, and complete the hardening by keeping them in a two per cent solution of ammonium-bichromate for four or five weeks Preserve them in spirit until required for section

METHOD OF CUTTING SECTIONS OF THE SPINAL CORD

Employ some form of freezing microtome Steep a piece of the cord, hardened by any of the above methods, but preferably by (*a*), in water for twenty-four hours, to remove all traces of the hardening agent, or the spirit Soak it in syrup (two ounces of sugar to

one ounce of water) for twenty-four or thirty-six hours, and then transfer it to a solution of gum for twelve hours, and then cut it in the ordinary way with a freezing microtome

Stain the sections with logwood, carmine, or aniline blue-black, and mount them in dammar

When investigating the structure of the nervous system, it is sometimes of importance to have a section only *partially* cleared up This may be done by examining a section in Farrant's solution, though, for general purposes, mounting in dammar is to be preferred

Make transverse sections through the hardened cervical portion of the cord of a cat, rabbit, or dog Stain a section with carmine, or logwood, or preferably with aniline blue-black one per cent solution, which stains the nerve-cells beautifully (p xliv) Mount the sections in dammar

Transverse Section of the Spinal Cord **EXAMINATION** (L)—Observe the *pia mater* investing the cord It consists of an outer part and an inner part, which latter sends numerous processes or septa into the cord Trace the passage of the outer layer into the anterior median fissure, and a process of the inner layer into the posterior median fissure

Observe that the cord is essentially a bilateral, symmetrical organ, its two halves being connected by a narrow bridge of tissue containing a small opening—the central canal Note the broad, shallow, and well-defined *anterior median fissure*, and the narrow, deeper, and less perfectly defined *posterior median fissure*, containing a process of the pia mater, often carrying a blood-vessel

If the section has been made at the level of the origin of the nerve-roots, trace the fibres of the anterior roots of a spinal nerve, leaving the cord by several bundles, whilst those of the posterior root enter it as one bundle

White matter—Observe the white matter, composed of the cut ends of nerve-fibres of the cord placed externally, and how each half is divided into an anterior, lateral, and posterior column by the above-described arrangement of the nerve-roots The posterior column is again divided into two (in the cervical region), by a narrow band of connective tissue, the narrow inner part, which lies next the posterior median fissure, is known as the fasciculus cuneatus

Grey Matter—Observe the grey matter more deeply stained, and lying in the form of a crescent in each half of the cord Its extremities constitute the anterior and posterior horns or cornua, though there is no well-marked limit between them Note in the anterior horn the numerous multipolar nerve-cells deeply stained, and observe the oval cap of deeply stained matter—the *substantia gelatinosa*—which covers the posterior cornua It is probably non-nervous in its nature, and consists of a peculiar accumulation of 'neurogleia' Observe the bridge of tissue connecting the two halves of the cord, and in it the *central canal*, lined with columnar ciliated epithelium,—in front and behind the canal, the anterior and posterior grey commissures, also the nerve-fibres crossing in front of the anterior grey commissure, forming the anterior white commissure (*Indicate the general arrangement in* Pl XXIII, Fig 1)

White Matter (H)—Observe the pia mater sending in septa, trace these septa, becoming continuous with a fine fibrous network—the *neuroglia*—lying between the nerve-fibres of the white substance Lying in the meshes of this network observe the cut ends of the nerve-fibres They are of different sizes some are large, others of medium size, and some are very small Each axis-cylinder is deeply stained, and is surrounded by a clear, non-stained area, which represents the white substance of Schwann, or myeline In sections which have been hardened for a long time in chromic acid, a well-marked concentric striation of the medulla

may be observed (see **Nerve**, p 42) (*Indicate the nerve-fibres in one half and the neuroglea in the other half of* Pl XXIII, Fig 2)

Neuroglea (H)—This is the delicate framework which supports the nerve-elements—fibres and cells—within the cord and brain It fills all the interstices between the nervous elements of the cord It consists of a semi-fluid substance—the neuroglea matrix—in which are imbedded numerous very fine neuroglea fibrils, which unite to form a network In it are also found many nucleated, branched, connective-tissue corpuscles The fibres seem to be allied in their nature to elastic fibres

Grey Matter (H)—Observe the large, nucleated, multipolar nerve-cells in the *anterior horn*, four or five branching processes may be traced from them for a considerable distance Each cell contains a distinct spherical nucleus, enclosed in a distinct membrane and a nucleolus Round each cell is a space—the lymph-space These cells are aggregated into three groups—an anterior, lateral, and median Study the *posterior horn*, and notice that the cells there are much smaller in size and fewer in number Notice the fibrillar arrangement of the rest of the grey matter, and study the substantia gelatinosa of Rolando (p 97), which is composed of a mass of neuroglea (*Indicate the anterior horn, with its nerve-cells, in* Pl XXIII, Fig 3)

Study similar sections made from the dorsal and lumbar regions of the cord, and compare them with the above Observe that the sections of the dorsal region are smaller than the others Compare the absolute amount of grey matter, and observe that it is greatest in the lumbar and cervical regions The amount of white matter is least in the lumbar and dorsal regions, and greatest in the cervical In the upper dorsal region notice a group of cells in the grey matter, midway between the anterior and posterior cornua—the *tractus inter-medio-lateralis*

Make similar sections of the spinal cord of an ox or man, or, best of all, that of a horse

It is necessary to *trace the course of the fibres* as seen in a section Observe (L) the *anterior root of a spinal nerve* Trace some of its fibres They enter the anterior cornu of its own side, and some of the fibres pass through the anterior white commissure to the anterior column of the opposite side Others turn into the lateral column on the same side Trace the fibres of the *posterior root* entering as a single bundle through the substantia gelatinosa

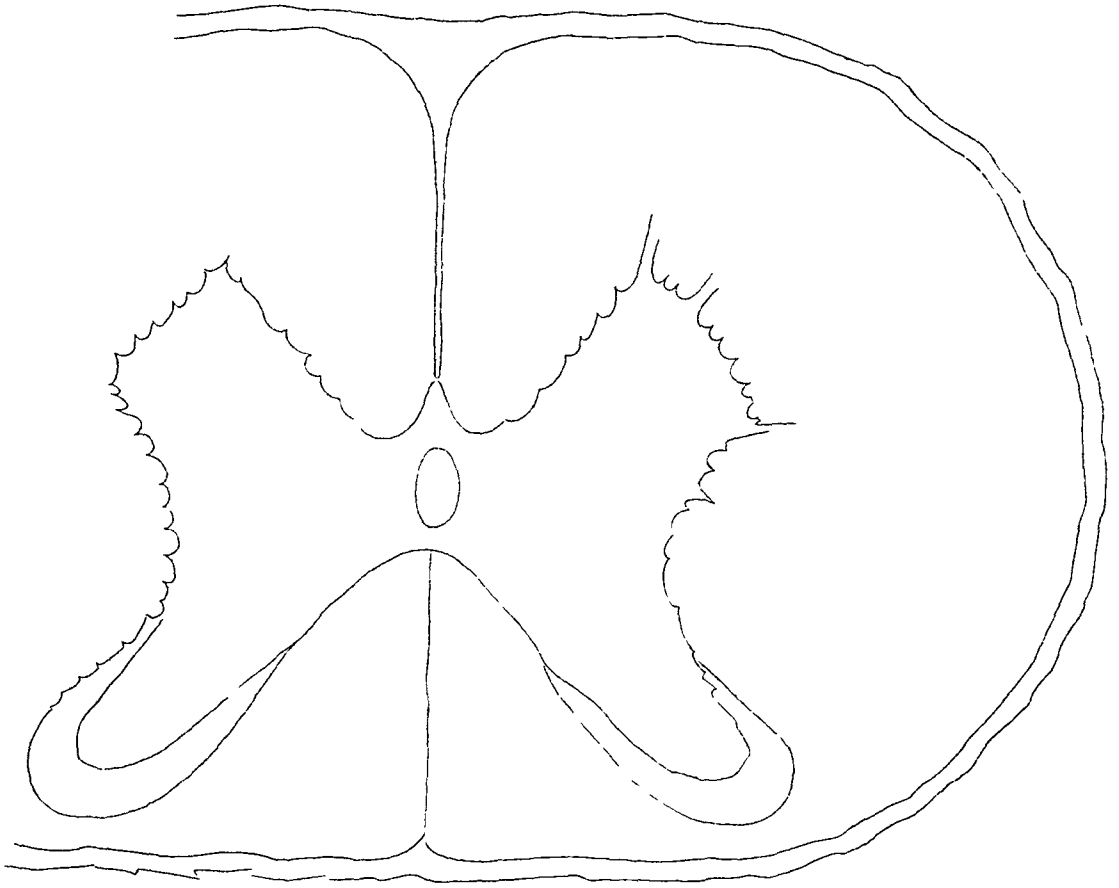
To study further the course of the nerve-fibres it is necessary to make antero-posterior sections through the grey and white matter This is best done in the cervical region of the cord of an ox Stain, and mount in the same way

Longitudinal Section of the Spinal Cord **EXAMINATION** (L)—Observe the grey matter in the centre with a band of white matter—the anterior and posterior columns cut longitudinally—on each side of it Trace the vertically-arranged nerve-fibres in these, and note the somewhat oblique entrance of the anterior roots, some of the fibres ascending, and others descending in the grey matter In the anterior cornu note the groups of multipolar nerve-cells and also the gelatinous substance in the posterior column and the entrance of a posterior root

TO ISOLATE A MULTIPOLAR NERVE-CELL OF THE SPINAL CORD

PREPARATION (*a*) **Dilute Potassic Bichromate**—Macerate a small piece—an eighth of an inch long—of the perfectly fresh spinal cord of an ox or sheep in a large quantity of a one-eighth per cent solution of potassic bichromate for two or three days After this time, cut out with scissors a small piece of the anterior horn, and tease it on a slide in water Stain the piece with carmine, and then continue to tease it with needles until isolated cells are

PLATE XXIII SPINAL CORD



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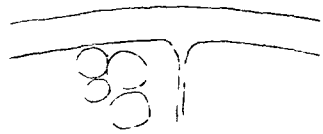

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obtained The process of teasing is best accomplished with the aid of a dissecting microscope, but the preparation ought to be examined from time to time with a low power A hair or thin piece of paper ought to be placed under the cover-glass, to prevent the cells from being displaced

(*b*) **Injection of Osmic Acid** —By means of a subcutaneous syringe, provided with a very fine gold nozzle, inject forcibly a small quantity of a half per cent solution of osmic acid into the anterior horn of a perfectly fresh spinal cord This serves to separate the parts, and at the same time to fix them Macerate the part for two days in dilute alcohol, and isolate the cells by carefully teasing out a piece, as described under (*a*)

(*c*) **Dilute Osmic Acid** —Macerate pieces, the size of half a pea, of the grey matter of the anterior horn of a perfectly fresh cord in a considerable quantity of a tenth per cent solution of osmic acid for ten days Wash away the greyish deposit, and place the pieces in a mixture consisting of equal parts of glycerine and water, for ten days or a fortnight Tease a small piece in glycerine The cells are easily stained with a very dilute solution of magenta

EXAMINATION (L and H)—Observe the large multipolar cells, with many branched processes One process—the axis-cylinder process, which is directly continuous with the axis-cylinder of a nerve-fibre—is always unbranched Observe the large nucleus, and note the longitudinal striation of the cell-substance and that of the axis-cylinder process

BLOOD-VESSELS OF THE SPINAL CORD

An entire animal must be injected, and sections made of the injected cord The grey matter is much more vascular than the white The sections are mounted in dammar, and need not be stained

The **connective tissue** of the subarachnoid space has already been referred to (p 23)

THE MEDULLA OBLONGATA

PREPARATION —The same methods as are employed for the spinal cord are to be used Make transverse sections of the human medulla, some through the decussation of the anterior pyramids, and others through the floor of the medulla and the olivary bodies Stain them with aniline blue-black, and mount in dammar

EXAMINATION (L) **Transverse Section through the decussation of the Pyramids** —Observe the crossing of the fibres from one side to the other , notice the enlarged posterior cornua (tubercles of Rolando), the central canal nearer the surface posteriorly

Through the Olivary Body —Observe the median raphe , the central canal has now expanded into the floor of the fourth ventricle Study the columns of the medulla from the centre in front outwards as anterior pyramids, olivary body, restiform body, and posterior pyramid on each side Notice the folded sheet of grey matter—*corpus dentatum*—in the olivary body, and in it notice the multipolar nerve-cells Observe the masses of grey matter in the floor of the fourth ventricle

THE CEREBELLUM

PREPARATION —The same methods are applicable as those detailed for the spinal cord Harden pieces—three-quarters of an inch square—of a human cerebellum in the Muller's fluid and spirit mixture (p xxxii), and proceed just as for the spinal cord If a human cerebellum cannot be obtained, use that of a cat, dog, or rabbit Make sections across the folia or leaflets Stain some with logwood, but preferably with aniline blue-black, and mount them in dammar

Vertical Section of the Cerebellum **EXAMINATION** (L)—Observe the folia or leaflets, with secondary leaflets on them Notice the dark-stained grey matter outside, and the lighter white matter inside The *grey matter* is covered with pia mater, which sends processes carrying blood-vessels into the substance of the organ It consists of (1) a broad, *outer grey* or *molecular layer*, containing at its inner part a single row of pear or flask-shaped cells, (2) the *cells of Purkinje*, which give off a number of branching processes, running outward, inside this is (3) the *inner granular* or *nuclear layer*, about one-third the breadth of (1) Observe the large number of small deeply stained granules, arranged in many rows, and then follows the white matter, consisting of nerve-fibres arranged more or less parallel to each other (*Indicate the layers in* Pl XXIV, Fig 1)

(H) (1) The *outer* or *cortical layer* contains, as a ground-work, a network of delicate nerve-fibrils, and mixed with these are the dichotomously divided branches of Purkinje's cells It contains a few multipolar ganglion-cells (Sankey), which seem to be connected with these processes

(2) *Purkinje's cells* occur in a single layer They are of a pear or flask-like shape Each cell has a single, unbranched, or axial cylinder process, which passes centrally, whilst from its outer surface it gives off a branched process, which splits up rapidly into branches or processes which divide again and again They can be traced nearly to the surface of the organ

(3) The *nuclear layer* is a broad layer, and contains a great number of deeply-stained spherical nuclei, whose exact nature is unknown Between them is a network of minute fibrils, which are probably nervous in their nature (*Indicate these layers in* Pl XXIV, Fig 2)

In the white matter note the small nerve-tubes, and between them the neuroglia, containing rows of small nucleated cells

Trace a *blood-vessel* in the grey matter, and observe that each one is surrounded by a narrow space—the perivascular lymph-space (His)

DOUBLE-STAINING OF THE CEREBELLUM

(1) **Eosin and Logwood** —Stain a section in a very dilute watery solution of eosin (p xlv) till it has a faint red colour This it does in a few seconds *Great care must be taken not* to overstain the preparation This forms an excellent ground-colour Wash the section in water, and then stain it with logwood, and mount it in dammar The cortical layer is reddish, and so are the cells of Purkinje, while the nuclear layer, and all other nuclei, have a logwood tint

(2) **Picrocarmine and Iodine-green** —Stain a section with picrocarmine Wash it in water

acidulated with dilute acetic acid, and, after washing in pure water, stain it with iodine-green, and mount it in dammar The outer layer and Purkinje's cells of the grey matter are stained red, and the inner layer is stained green

THE CEREBRUM

PREPARATION—Prepare it in the same way as the cerebellum (p 100), using the same precautions both for hardening the tissue and for cutting it as indicated for the spinal cord (p 96) Select a frontal convolution of a human brain for examination Stain the sections with aniline blue-black, and mount them in dammar

Vertical Section of a Human Cerebral Convolution **EXAMINATION** (L)—Observe the shape of a convolution, and contrast it with that of the cerebellum Notice the pia mater surrounding the convolution, and sending fine processes carrying blood-vessels into the cortex Notice the deeply stained grey matter or cortex, and the white matter within Examine the layers of the grey matter from without inwards It is possible to distinguish five layers, varying in thickness and structural characters (Meynert) These layers are not accurately mapped off from each other, still they can be seen It is to be remembered, however, that there are variations in different parts of the cortex In some places only four layers can be distinguished

1 The outer layer contains few cells of any kind imbedded in a clear matrix It occupies about one-tenth of the entire thickness of the grey matter

2 This is about equal in thickness to the preceding, though it is easily distinguished by its containing a large number of densely packed small pyramidal cells Owing to the large number of cells in this layer it always appears deeply stained

3 This layer is much wider, and, owing to the cells occurring in it being placed well apart from each other, it appears somewhat lighter than the second layer It consists of large and small pyramidal cells, so placed that their apices are directed towards the surface of the convolution, and their bases towards the white matter

4 This layer contains small irregular corpuscles with few processes

5 This layer is broader than the one above it, and contains similar corpuscles mixed with a few fusiform cells At its lower part it gradually shades into the *white matter*, which consists of very fine medullated fibres, supported by neuroglia (*Indicate the general arrangement of the cells in* Pl XXIV, Fig 3)

(H) Study in detail the various layers, and especially note the cells of the third layer The cells are distinctly pyramidal Trace the long, branching process directed towards the surface, and the branching processes from each angle of the base Each cell has a central axial cylinder-process Each ganglion-cell lies in a lymph-space It is to be observed, however, that these cells only appear pyramidal when the plane of the section passes parallel to their long axis If cut transversely they appear triangular (*Indicate a nerve-cell in* Pl XXIV, Fig 4)

Select a *blood-vessel* of the grey matter, and observe its perivascular lymph-space

The processes of the cells are best seen in preparations which are only *partially* cleared up under the influence of clove oil This is a most important method of investigation I have often seen in this way delicate fibrils, not unlike elastic fibres, and which are not distinct when the section is completely cleared up

BLOOD-VESSELS OF THE BRAIN

The entire animal must be injected Make vertical sections and mount them in dammar Observe the greater vascularity of the grey matter

NERVE-GANGLIA GASSERIAN OR SPINAL GANGLIA

PREPARATION —With a saw divide longitudinally the head of a sheep just killed With a scalpel remove the Gasserian ganglia from the apex of the petrous part of the temporal bone, and place them in the chromic acid and spirit fluid for eight days Make longitudinal and transverse sections Stain them with logwood and mount them in dammar, or in Farrant's solution The ganglion on the posterior root of a spinal nerve of a cat or dog may be hardened by the same method

Longitudinal Section of a Spinal Ganglion EXAMINATION (L) —Observe the sheath of the ganglion—within it are numerous large ganglionic cells—and note their arrangement Observe strands of nerve-fibres sweeping through the ganglion, and processes of connective tissue passing in from the capsule into the ganglion, and supporting the cells and nerve-fibres (*Indicate the nerve-cells and fibres in* Pl XXIV, Fig 5)

(H) Observe the nerve-cells, with their well-marked sheath or capsule lined with squames, the nuclei of which are well-marked, the granular mass of the cell, containing a round eccentric nucleus with an envelope, and a distinct nucleolus It is difficult to recognise the processes of the cell itself Note the medullated nerve-fibres

In the examination of the transverse sections note the cells as before, but between them bundles of medullated nerve-fibres cut transversely

It is also desirable to examine the cells from a fresh Gasserian ganglion For this purpose tease a small piece of the fresh Gasserian ganglion of a sheep in a very small quantity of salt solution, and stain it with magenta solution Details similar to those described above are seen on examination, though the processes of the cells are generally broken off, and the cells themselves are frequently dislocated from their capsules (*Indicate an isolated cell within its capsule in* Pl XXIV, Fig 7, *and without its capsule in* Fig 6)

GANGLIA OF THE SYMPATHETIC NERVOUS SYSTEM

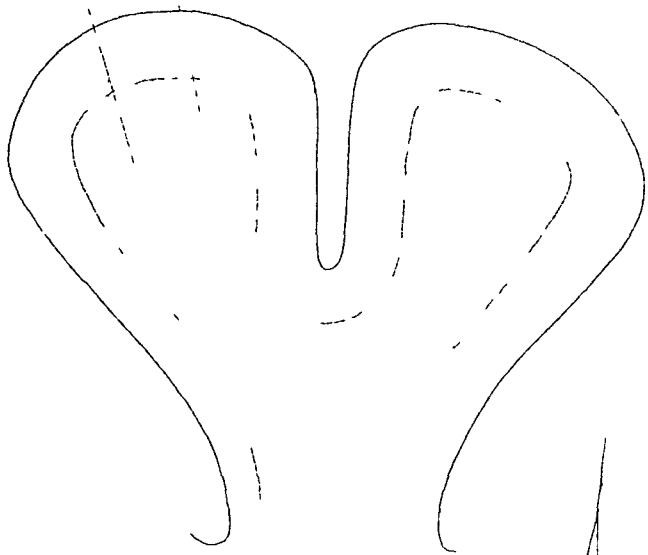
The non-medullated nerve-fibres have already been examined (p 45) The nerve-cells of the sympathetic system have still to be considered

PREPARATION —Remove a sympathetic ganglion from the thorax of any animal, or preferably use the human superior or inferior cervical sympathetic ganglion Harden it in Muller's fluid, or the chromic acid and spirit fluid, for four or five days, and make transverse sections Stain sections with logwood, and others with picrocarmine, and mount them in Farrant's solution

EXAMINATION (L) —Observe the capsule of the ganglion composed of fibrous tissue, and notice the cells and their arrangement Place a cell in the field of the microscope

(H) Observe the cell-capsule, with its included nucleated cell, which gives off processes

PLATE XXIV BRAIN & NERVE GANGLIA



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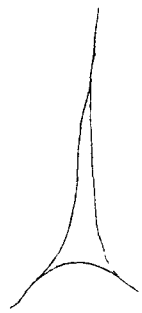

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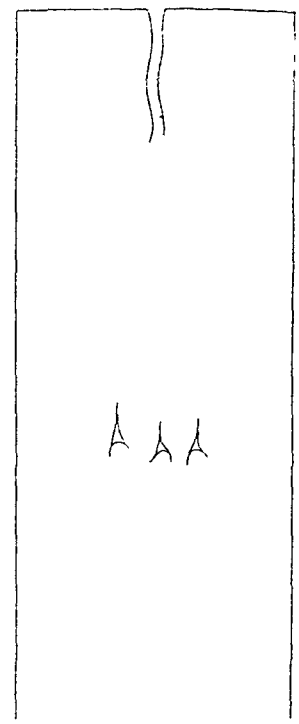

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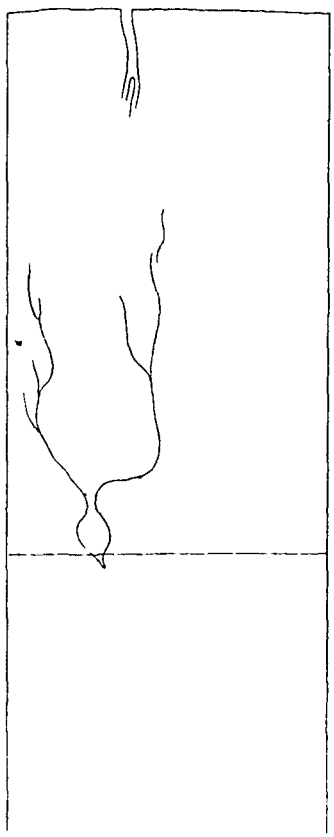

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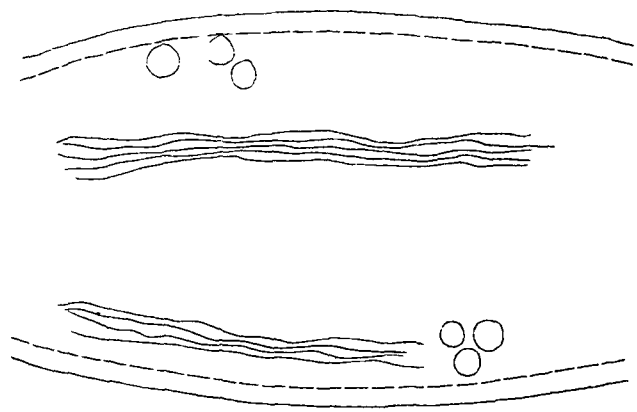

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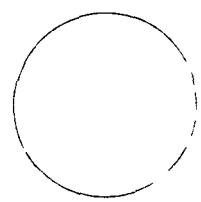

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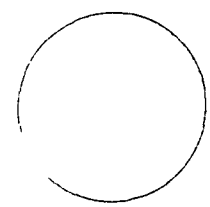

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Minter Bros lith

that pierce the capsule The cells very frequently contain a yellowish-coloured pigment Between the cells will be found transverse sections of non-medullated nerve-fibres

SYMPATHETIC NERVE-CELLS OF THE FROG

These have already been alluded to in connection with the heart They occur in large numbers along the course of the abdominal aorta in the frog, and are easily demonstrated by the lime-juice and gold method (p xlv) They may also be obtained from the sympathetic ganglia It is easy to demonstrate the straight process, but by no means so easy to see the spiral one

Isolation of Sympathetic Nerve cells—Adopt the same methods as are recommended for the nerve-cells of the spinal cord

THE EYE

THE CORNEA

PREPARATION—Cut out the fresh cornea from the eye of a cat or rabbit In excising the eyeball, be careful not to squeeze or injure it For the removal of the cornea, push a narrow knife into it at its junction with the sclerotic, and then cut it out with scissors Harden it in a two per cent solution of potassic bichromate for ten days, or in the chromic acid and spirit mixture for a week Make vertical sections, stain some with logwood and others with picrocarmine, and mount in Farrant's solution, and also in dammar

EXAMINATION (L)—Observe the epithelium occurring in many layers—*conjunctiva corneæ* or *anterior epithelium*—covering the anterior surface of the cornea proper In the human cornea, and in the cornea of some animals, the epithelium rests on a narrow, elastic, transparent membrane—the *anterior elastic lamina* or *Bowman's membrane*—but it is either absent or but feebly developed in the cornea of the cat and dog It seems to differ from the next layer chiefly in the absence of corneal corpuscles The substance forming the cornea proper—*substantia propria*—is stained red, and on its posterior surface there is a section of a clear, sharply-defined membrane stained yellow—the *membrane of Descemet*, or *posterior elastic lamina*—and covering the posterior surface of this membrane there is a single layer of flattened nucleated epithelial cells seen in profile Examine each of these parts (*Indicate the general arrangement in one half of* Pl XXV, Fig 1)

(H) The **anterior epithelium** Begin at the surface, and note the flattened cells seen in section The deeper layers consist of two or three layers of polyhedral cells, each with a spherical nucleus They are so arranged as to fit into each other, and the deepest layer resting on the cornea is composed of a row of columnar cells placed perpendicularly Observe how processes of the cells in the layer above this fit into depressions between the apices of the columnar cells (Cleland and Stirling) The columnar cells are not all of the same height, and their lower end possesses a flat expansion or foot-plate (Lott and Stirling), which rests on the cornea Prickle-cells are seen in the middle layers, especially where the epithelium is in very many layers, as in the cornea of the ox (See p 9)

The **anterior elastic lamina** is a homogeneous, transparent membrane devoid of cornea-corpuscles It is perforated here and there by a few oblique channels, which transmit fine nerve-fibrils—the *rami perforantes*

The **substantia propria** is composed of a series of layers of fibrillar connective tissue, arranged in the form of lamellæ, placed one outside the other, and parallel to the surface of the cornea These lamellæ, and also the fibres which compose them, are held together by an albuminous cement-substance Bundles of fibrous tissue pass from one lamella to another Near the anterior surface a few fibres perforate the lamellæ obliquely, and constitute the

fibræ arcuatæ Note the edges of these lamellæ seen in section In the ground-substance which separates two adjoining lamellæ, there exists a series of lacunæ or cell-spaces of irregular branched form, which communicate freely by means of fine channels with lacunæ in the same plane, and also with the lacunæ lying above and below them This is the lymph-canalicular system of V Recklinghausen Each lacuna contains a nucleated cell-plate or *corneal corpuscle*, which does not completely fill the space, so that lymph and colourless blood-corpuscles can move between it and the wall of the space These corpuscles are seen as flat, narrow, nucleated, stained spindles lying between the lamellæ in oval spaces, which they do not fill completely Each corpuscle sends processes into the canaliculi, so that they anastomose to form a network They cannot be seen in this preparation , it requires the use of gold chloride to show their processes satisfactorily (p 105)

The **posterior elastic lamina** is a structureless elastic membrane with sharply defined margins, which stains of a deep yellow tint with picrocarmine It may become detached, when it curls up It consists of fibrils Study the single layer of polyhedral, nucleated, granular, *endothelial cells* on its posterior surface (*membrana Descemeti*)

CORNEA-CORPUSCLES, AND NERVES OF THE CORNEA

PREPARATION —These are both demonstrated by the same method, viz the **gold method** This may be accomplished in several ways

(*a*) **Reduction by Dilute Acetic Acid** —Remove the cornea from an animal just killed (*e g* cat, rabbit, or guinea-pig), using the same precautions to avoid stretching it as indicated at p 104. Place it in a few c c of a half per cent solution of gold chloride, and keep it in the dark Let it remain in the gold for half an hour to an hour in the case of the cornea of a guinea-pig, and an hour and a half to two hours in that of a rabbit Remove it, and wash it in distilled water, and place it in water slightly acidulated with acetic acid, and expose it to the light until it becomes of a decidedly purplish or slate-grey colour throughout Make vertical and horizontal sections, and mount them in glycerine

(*b*) **Reduction by strong Tartaric Acid** —Proceed as before up to the stage where the cornea is washed in distilled water Expose it for a day, or two days, to the light in distilled water without the addition of any acid, until it assumes a violet colour Place it in a mixture of glycerine, one part, and water, two parts, for two or three days, and keep it in the dark Wash, and place it in a small beaker half filled with a nearly saturated watery solution of tartaric acid Heat this in a water-bath kept about 45° C until it assumes a deep purple colour, which it does in a few minutes Make sections—vertical and horizontal—and mount them in glycerine

(*c*) **Reduction with Formic Acid.**—Place the cornea in the juice of a fresh lemon for five minutes Wash it well, and transfer it to a one per cent gold chloride solution for half an hour Wash it again, and place it in a twenty-five per cent solution of formic acid for twenty-four hours It must be kept in the dark until the reduction is complete This process removes all the epithelium, and is, therefore, not suited for showing the terminations of the nerve-fibrils between the cells of the anterior epithelium, but it shows the nerve-fibrils and corpuscles in the cornea admirably

Vertical Section of Cornea **EXAMINATION** (L and H) —Observe the same general arrangement of parts as before, but note the rows of connective-tissue corpuscles, stained of a deep

reddish purple, and lying between the laminæ of the cornea Trace some processes communicating with the cells in the same plane, and others with processes of those cells that lie above and below them (*Indicate this in one half of* Pl XXV, Fig 1)

Terminations of the Corneal Nerves—The nerve-plexus in the cornea can be well studied in this section Observe the primary nerve-plexus in the corneal tissue, and from it trace bundles of nerves running vertically to form the sub-epithelial plexus It is difficult to find the very fine fibrils which proceed from this plexus to end—as an epithelial plexus—between the cells of the anterior epithelium

Horizontal Section of the Cornea **EXAMINATION** (L)—Observe the branched corneal corpuscles seen on the flat They are deeply stained, so they stand out distinctly The primary nerve-plexus in the cornea will be only just distinguishable

(H) **Cornea Corpuscles**—Note their flattened characters, their branched processes, and their frequent anastomoses with processes from adjoining cells Each one consists of a 'granular' plate with a nucleus (Pl XXV, Fig 2)

Nerve-Plexus—Observe a meshwork of fine nerve-fibrils, with perhaps varicosities on them It may be that a bundle of nerve-fibrils, constituting an axis-cylinder, and surrounded with a primitive sheath, may be found

THE CELL-SPACES OF THE CORNEA

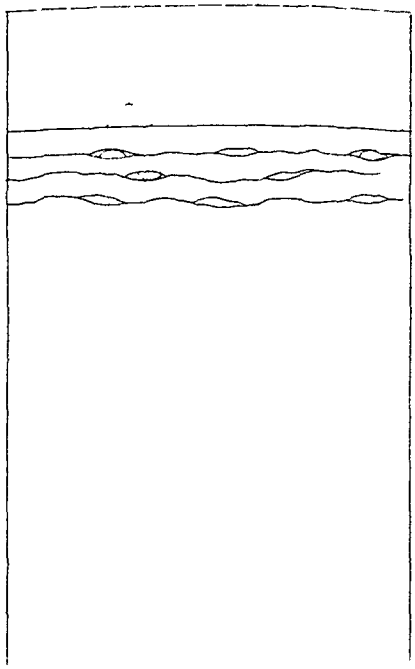
PREPARATION—Pith a frog, and scrape away the epithelium from the anterior surface of the cornea Drop into the eye a few drops of a two per cent solution of silver nitrate Leave the silver to act until the cornea becomes of a greyish-white colour throughout, which usually takes from fifteen to twenty minutes Excise the eyeball, and cut out the cornea, and make one or two cuts in its margin, and lay it flat on a slide in a drop of glycerine, and expose it to the light until it becomes brown in colour Similar preparations may be made from a mammal, but of course the animal must be deeply anæsthetised to avoid all pain

EXAMINATION (L and H)—The cement-substance is stained brown, and in it is left a series of clear branched and anastomosing spaces—the cell-spaces or lymph-canalicular spaces They correspond exactly in shape to the corneal corpuscles, which partially fill them (Pl XXV, Fig 3) The spaces appear to be empty, because the cells filling them are not affected by the silver, but if a section be stained with logwood the nuclei of the corpuscles are brought into view Double-staining with silver and then gold brings the corpuscles into view The silver gives the negative picture and the gold preparations already described represent the positive picture

The Fibrous Tissue of the Cornea **PREPARATION**—Place a small piece of the cornea of any newly-killed animal in a solution of picric acid for twenty-four hours Tease a small piece in water or glycerine

EXAMINATION (H)—Observe the bundles of wavy fibrous tissue stained yellow This preparation need not be kept

PLATE XXV CORNEA LENS & CHOROID



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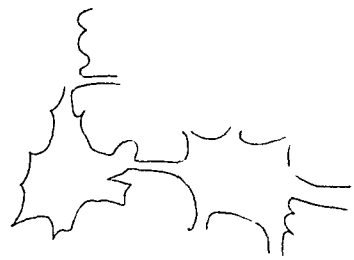

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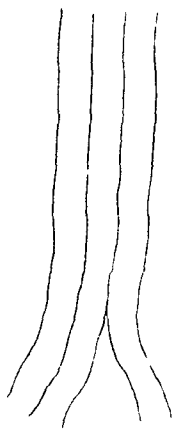

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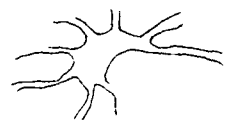

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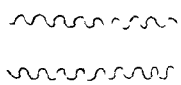

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THE SCLEROTIC

The sclerotic consists of bundles of fibrous tissue crossing each other in all directions in the plane of the membrane These bundles become continuous with the substantia propria of the cornea at its margin Mixed with them is a small quantity of elastic tissue Sections may be made from any eye which has been hardened in Muller's fluid, or chromic acid and spirit fluid Stain the sections with logwood and mount them in dammar

THE CRYSTALLINE LENS

PREPARATION (*a*) **Boiling**—Boil the lens of a fish—*e g* cod—for a few minutes, until it becomes white Peel off the outer softer part of the lens, and tease a little of the deeper, less brittle part, in Farrant's solution

(*b*) **Glycerine and Nitric Acid Fluid**—Place the eyeball of a frog, or the lens of any other animal, in the above fluid (p xxxiv) for twenty-four hours, and then in water for the same time This dissolves all the cement, and renders the lens-fibres so tough that they do not break readily when they are teased Tease a small piece in glycerine as above

Make a similar preparation of the lens of a dog, cat, or rabbit, and note that the lens-fibres in the case of these animals are not nearly so markedly serrated (Pl XXV, Fig 4)

EXAMINATION (H)—Observe the lens-fibres of a codfish They are long, narrow bands, with well-marked teeth or serrations along their narrow margins, the teeth of contiguous fibres dovetailing with one another (Pl XXV, Fig 5)

(*c*) **Potassic Bichromate**—Transverse sections ought to be made across a lens hardened in one per cent potassic bichromate solution for a week Mount them in Farrant's solution They are very apt, however, to fall to pieces The fibres are hexagons when seen in transverse section

The **capsule of the lens**, and the layer of epithelium lining it, are easily obtained from the eye of a frog

The arrangement of the lens-fibres is easily made out in sections of a fœtal eye

THE CHOROID

Sections may be made through the coats of the eyeball of an ox, hardened in chromic acid and spirit mixture, to include the sclerotic and the choroid It consists of bundles of connective tissue, with some elastic fibres, many blood-vessels and branched pigment-cells The layer of hexagonal pigment-cells occurring on its inner surface is now recognised as belonging to the retina (Pl XXV, Fig 7)

Pigment-Cells of the Choroid. PREPARATION—From the eye of an ox, hardened as described for the ciliary muscle, remove the retina. Scrape off a little of the dark pigment-layer which adheres to the sclerotic, and diffuse or spread it out in glycerine Cover

EXAMINATION (H)—Observe the branched flattened cells, like branched connective-tissue

cells The nucleus usually does not contain pigment, though the rest of the cell and its processes are loaded with fine granules (melanin) of a brown or black colour (Pl XXV, Fig 6)

THE CILIARY MUSCLE

PREPARATION—Divide the eyeball of an ox or sheep, with a sharp razor, transversely, half an inch behind the circumference of the cornea, and remove the lens, but be careful to retain the choroid and iris Harden the anterior half of the eyeball in the chromic acid and spirit fluid for eight days Make horizontal sections, to include the sclerotic, the cornea, and the iris Stain a section with picrocarmine or logwood and mount it in Farrant's solution or dammar

EXAMINATION (L)—Observe the cornea in the front part continuous with the sclerotic behind At the line of junction notice the section of the pigmented iris projecting inwards Trace the choroid posteriorly, lining the sclerotic Observe the fibres of the ciliary muscle arising at the junction of the sclerotic and cornea, and passing backwards over and outside the choroid, into which it is inserted Sections of the ciliary processes may be seen Trace the membrane of Descemet of the cornea backwards, and notice that at the junction of the cornea and sclerotic it splits into fine transparent bundles, some of which curve round towards the iris, whilst others spread out like a fan over the ciliary processes, thus forming the *ligamentum pectinatum iridis* At the junction of the cornea and sclerotic notice a small aperture This is a section of the venous sinus, or *canal of Schlemm* (Pl XXVI, Fig 4)

RETINA

PREPARATION **Muller's Fluid and Spirit**—It is desirable to use the retinæ of several animals, because each shows some special feature

(*a*) With a sharp razor divide the eyeball of a pig, cat, and ox transversely, and place the posterior halves in a mixture of three parts of Muller's fluid and one of spirit, and keep the preparations in the dark and in a cool place for a week or ten days Make vertical sections of each of the above in the ordinary way, by means of a freezing microtome, after the tissues have been soaked in syrup and then in gum, as described at p xxxix This is a most important precaution to ensure good preparations Make the sections through the sclerotic, choroid, and retina, though the retina is very apt to separate from the other coats Stain a section of each, with either logwood or picrocarmine Mount them in Farrant's solution This method shows the nerve-elements best

(*b*) **Chromic Acid and Spirit Mixture**—This may also be used, and shows the connective-tissue elements best, besides, it does not make the retina quite so brittle as the Muller's fluid

(*c*) **Osmic Acid**—Place the retina of a frog and that of a fish—*e g* cod—in a few cubic centimètres of a quarter per cent solution of osmic acid for three or four hours The retinæ are rapidly blackened and 'fixed' Soak the retinæ for several hours in water to get rid of the surplus osmic acid, and tease a small part of each in a drop of glycerine, and cover

Vertical Section of the Retina of a Cat or Pig **EXAMINATION** (L)—Observe from without inwards --

1 The uvea, or *pigment-cells of the retina*, sending fine processes into the next layer This layer is often detached, and was formerly regarded as belonging to the choroid

2 The *layer of rods and cones* (Jacob's membrane) The rods are easily detected in groups, and seen to be more numerous than the cones, which are much shorter, and not so easily seen

3 At the lower end of the rods and cones a well-defined line, indicating the position of the *external limiting membrane*

4. The *outer nuclear layer*, deeply stained—red or blue—consisting of spherical nuclei arranged in several rows It is thicker than the inner nuclear layer

5 The *external granular* or *internuclear layer*, a narrow layer

6 The *inner nuclear layer*, also deeply stained—red or blue—and consisting of three or four rows of spherical nuclei, larger than those of the outer layer

7 The *inner granular layer*, much broader than the outer layer of the same name

8 The *cellular* or *ganglionic layer*, consisting of a single row of large, well-marked multipolar nerve-cells, each with a well-defined nucleus

9 The *layer of optic nerve-fibres*, merely indicated as fine threads, as they have no white substance of Schwann

10 Most internally is the *internal limiting membrane* From this at regular intervals arise, by a broad-winged base, the *fibres of Muller* (connective tissue), which proceed outwards in the retina between the nerve-cells They may be traced as far as layer number 6 In the innermost part of the retina, near the cellular layer, sections of large blood-vessels are to be found Each of these parts ought to be examined separately (*Indicate the general arrangement in* Pl XXVI, Fig 1)

(H) The **pigment-cells** or **uvea**—These cells are cut vertically, and are apt to be detached They send fine processes into the next layer

Rods and Cones—Observe the rods, more numerous than the short club-shaped cones Each consists of an outer and an inner segment Notice that the inner segment perforates the outer limiting membrane Study each of the layers in succession Notice especially the nerve-cells—their large size, their well-defined nucleus and nucleolus, the former with a distinct envelope around it, their processes—one central, which has a connection with one of the nerve-fibres of the optic nerve, and several processes which proceed outwards and branch in the inner granular layer Trace the outward course of the fibres of Muller

The retina of the pig is selected because it shows so well the rods and cones, but it is advisable to study also a section of the retina of an ox, because in it the ganglionic cells are specially large and easily investigated

Retina of the Frog in Osmic Acid **EXAMINATION** (H)—Tease out a small piece in glycerine The rods are very large, and so are easily seen Both rods and cones consist of an outer and an inner segment or member In the fresh state the outer member is transparent, bright and glistening, but it is rapidly blackened by the osmic acid, and so is easily recognised It not unfrequently shows a tendency to cleave transversely Each inner segment is pale, and more or less granular They may be seen perforating the external limiting membrane, and containing one of the nuclei of the external nuclear layer in their course (*Indicate the rods and these segments in* Pl XXVI, Fig 2)

The Retina of a Codfish. **EXAMINATION** (H)—This is specially selected, because it shows the cones so admirably Select a cone, and observe its large size, notice its short tapering outer segment and its large conical inner one, and notice how readily it shows the transverse

markings on the outer segment of the cone, sometimes it may be found split transversely A view of the cones imbedded amongst a great number of the narrow rods may be obtained from above (Pl XXVI, Fig 3) Many non-medullated fibres of the optic nerve will be found

DOUBLE-STAINING OF THE RETINA

Rosein and Iodine-green—Stain a section of the retina of a cat or ox in a solution of rosein (p xlv), and then in iodine-green Mount it in dammar The two nuclear or granular layers become of a greenish colour, and all the other parts are red

HEXAGONAL PIGMENT-CELLS OF THE RETINA

From an eye hardened in chromic acid and spirit scrape off a little of the black pigment lining the choroid A sheet of flattened hexagonal pigment-cells is easily obtained The nucleus is not pigmented (Pl XXV, Fig 7)

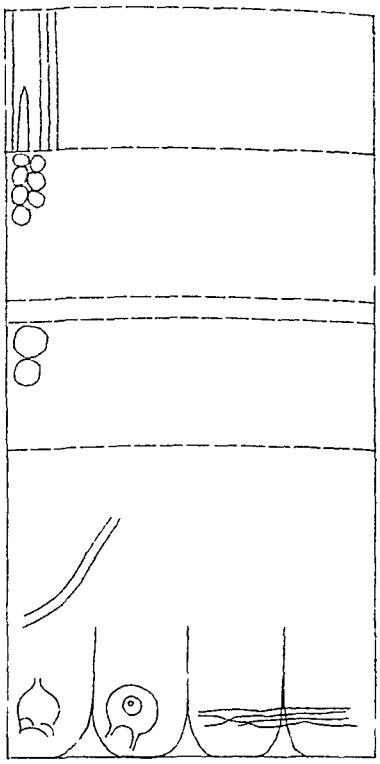
THE EYELIDS

The eyelids are prepared in exactly the same way as recommended for the lips (p 60)

THE LACHRYMAL GLANDS

Prepare them in the same way as the salivary glands, which they closely resemble in structure

PLATE XXVI RETINA & CILIARY MUSCLE



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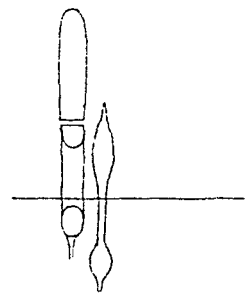

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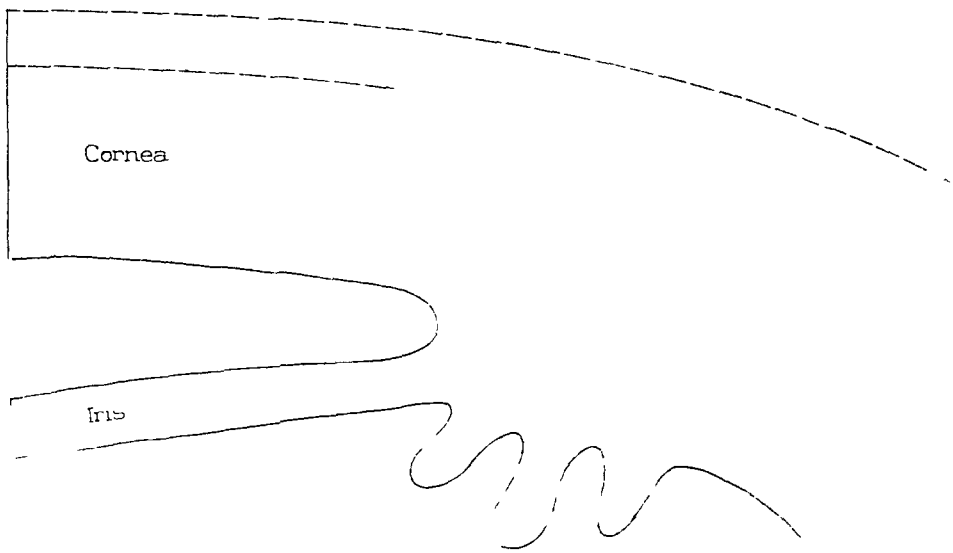

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THE NOSE.

(A) OLFACTORY EPITHELIUM OF THE FROG OR NEWT

PREPARATION (*a*) **Dilute Alcohol.**—Cut off the head of a newt or frog, and slit up the nostrils with scissors, place it in a small quantity of dilute alcohol for twenty-four hours Stain the whole head with picrocarmine, and tease a little of the stained epithelium from the olfactory region in a drop of glycerine This is the best method

(*b*) Maceration for two days, in a quarter per cent solution of potassic bichromate, yields fair results

(*c*) Maceration of the entire head in a half per cent solution of osmic acid for five hours, followed by steeping in water, also 'fixes' the cells

EXAMINATION (H)—Observe the ordinary columnar epithelial cells, devoid of cilia, but amongst these observe the narrow, delicate, spindle-shaped cells, each with a spherical nucleus These are the true *olfactory cells* To their free end a bunch of fine bristles is attached Their lower or fixed end consists of a very fine filament with varicosities on it It is directly continuous with the terminations of the fibres of the olfactory nerve The olfactory cells are richly scattered amongst the above-described cylindrical epithelium (*Indicate these cells in* Pl XXVII, Fig 2)

(B) Similar preparations may be made of the olfactory cells from the olfactory region of the nose of a mammal In this case the olfactory cells are devoid of bristles on their free ends

(C) OLFACTORY EPITHELIUM OF A MAMMAL

PREPARATION—Divide, by means of a saw, the head of a freshly-killed rabbit, dog, guinea-pig, or sheep in a longitudinal direction, and parallel to the nasal septum Cut out the nasal septum so as to expose the olfactory region, easily recognised by its brown colour Harden this in Muller's fluid for two days, and then transfer it to the chromic acid and spirit mixture for a week Complete the hardening in weak, and then in strong, spirit Make vertical sections, and stain them with logwood, and mount them in dammar or Farrant's solution, or a double-staining of logwood and picrocarmine may be employed

EXAMINATION (L)—Observe the mucous membrane, covered by columnar epithelium The upper row of nuclei seen belongs to the supporting or columnar cells, the deeper row belongs to the true olfactory cells In the deeper layers of the epithelium, and also in the glands, are pigment-granules Sections of gland-ducts and alveoli, filled with granular-looking cells—Bowman's glands—are readily seen (H) Examine the individual parts (*Indicate the olfactory epithelium and Bowman's glands in* Pl XXVII, Fig 1)

TRANSVERSE SECTION OF THE NOSE

The student ought to have a section through the whole nasal chamber in order to study the respiratory mucous membrane, nasal septum, the turbinated bones, and the general arrangement and comparative vascularity of the parts

PREPARATION—Inject the blood-vessels of the head of a rabbit or guinea-pig with a carmine-gelatine mass from the aorta Open the skull, and remove the brain, disarticulate the lower jaw, and cut off the head Place it in Muller's fluid for three or four days, and then in the chromic acid and spirit mixture for the same time This fixes the various elements Place it in the chromic acid and nitric acid mixture, until the bones are completely decalcified Make sections across the entire nasal chamber in a freezing microtome Doubly stain them with logwood and iodine-green, and mount them in dammar These form most instructive preparations

THE EAR

THIS is the only organ of which one cannot give the student a section to carry away with him, on account of their difficulty of preparation, and also because a properly prepared cochlea yields but very few good sections

THE SEMICIRCULAR CANALS

These are best obtained from the skate, whose cartilaginous cranium should be cut away until the canals with their saccule and utricle are exposed The canals are hardened in chromic acid and spirit mixture for a week, and then transferred to spirit The ampullæ receive nerves and may be hardened in osmic acid Sections of these canals must be made with a freezing microtome after steeping in syrup and gum

THE COCHLEA

PREPARATION (A)—The guinea-pig is the best animal to employ Kill the animal and disarticulate the lower jaw, when the osseous tympanic bulla is exposed It lies just behind the fossa for the condyle of the lower jaw Cut away the external auditory meatus, and remove the petrous portion of the temporal bone with the bulla from the other bones of the skull Open the bulla with bone-forceps, when a conical elevation—the cochlea—is seen Remove all the surrounding bone, so as to isolate the cochlea Place it at once in Muller's fluid, to harden its delicate tissues, for a fortnight It is necessary, however, to remove the lime-salts from the bone, which is done by transferring the organ to a mixture of chromic acid and nitric acid (p xxxiii), or to a solution of picric acid The fluid ought to be shaken from time to time, which greatly facilitates the process of softening After softening preserve it, first in weak spirit, and then transfer it to strong spirit When sections are to be made it must be steeped in water, to get rid of the spirit, and then it is subjected to the syrup-and-gum process These substances support its delicate tissues, and sections must be made parallel with the modiolus, *i e*, across the turns of the cochlea, in a freezing microtome They must be manipulated with the greatest care, and ought to be stained with logwood or picrocarmine and mounted in glycerine

(B) If the human cochlea be employed, it should be obtained as fresh as possible Cut out the part of the temporal bone containing the cochlea, from the human subject Split this up in the axis of the meatus auditorius internus, this gives two pieces of bone, one containing the cochlea and a part of the vestibule, and the other the semicircular canals

and part of the vestibule Place the pieces at once in a mixture of three parts of Muller's fluid and one of spirit for two weeks After that transfer them to a quarter per cent solution of chromic acid for a fortnight At the end of that time add a few drops of hydrochloric acid to the fluid until the bone is softened The bone around the labyrinth is very dense, and requires a longer time than the other parts to become soft Wash it thoroughly for twelve hours in water, and place the whole cochlea for thirty-six hours in syrup and forty-eight in gum, and freeze it in gum in a freezing microtome

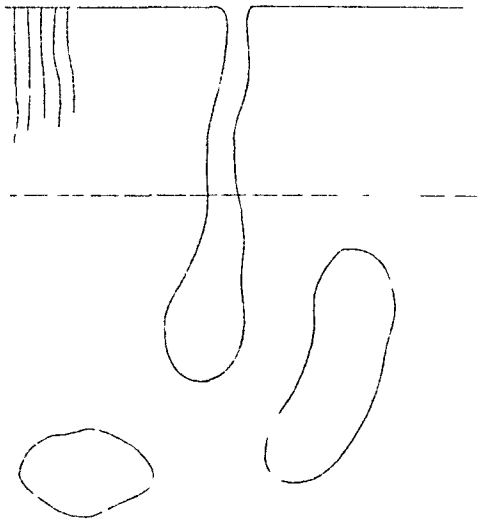
The student must examine a series of preparations prepared by the above methods

Vertical Section of a Cochlea **EXAMINATION** (L)—Observe the *modiolus* or central column in the centre, with the cochlear branches of the auditory nerve ascending within it Note a section of one of the turns of the cochlea Each turn is divided into two compartments by the *lamina spiralis* The upper compartment is called the *scala vestibuli*, and the lower one the *scala tympani* Note that the *lamina spiralis* consists of an inner osseous portion consisting of two plates of bone between which runs a branch of the cochlear nerve on its way to structures which rest on the outer or *membranous* part of the lamina In the course of the nerve observe the *spiral ganglion* The membranous part consists of a *basilar membrane*, on which rests the organ of Corti and certain epithelial cells which are connected with the terminations of the cochlear branch of the auditory nerve It is connected to the outer wall of the cochlea by the *spiral ligament*, which consists of a mass of connective tissue, continuous with the periosteum of the bone

Notice a thin membrane running obliquely upwards and outwards in the scala vestibuli, from the outer part of the lamina spiral ossea to the outer wall of the cochlea, this is the *membrane of Reissner*, which cuts off a narrow triangular channel, the *ductus cochleæ*, or scala media, from the scala vestibuli proper The floor of this narrow canal is made up of the outer part of the osseous lamina and the basilar membrane At the outer extremity of the osseous lamina is a spiral groove excavated like a C, with the concavity looking outwards, this is the *sulcus spiralis* The projecting upper edge of the groove is called the *crista spiralis* Observe the *organ of Corti*, consisting of an inner and an outer set of *rods* resting on the basilar membrane These two sets of rods incline towards each other, their heads touching, so as to leave a triangular passage or tunnel under them, which is filled with endolymph The outer rods are longer than the inner ones Internal to the inner rods and external to the outer ones are a number of epithelial cells, some of them with hairs attached to them—the *outer* and *inner hair-cells* A faintly-striated membrane springs from the upper surface of the lamina spiralis ossea, close to the crista spiralis, and may be seen partially to cover the organ of Corti It was called by Claudius the *membrana tectoria* It terminates over and is attached to the outer hair-cells The outer and inner hair-cells send prolongations through another membrane which covers them—the *membrana reticularis* (*Indicate the general arrangement in* Pl XXVII, Fig 3)

(H) The *membrane of Reissner* consists of a very delicate hyaline-membrane covered on each surface with a single layer of epithelium Its surface, directed towards the cochlear canal, is composed of polyhedral flattened cells, whilst the vestibular surface is covered by very thin endothelial cells The *membrana basilaris* has, on its upper surface, many epithelial cells, some of them specially to form the organ of Corti, and others in which the cochlear branch of the auditory nerve probably terminates, and which are, in fact, its 'end-organs' The basilar membrane consists of one or two layers of hyaline membrane, which are covered on the tympanic side by a single layer of endothelial cells On the upper or vestibular surface of the

PLATE XXVII NOSE & COCHLEA



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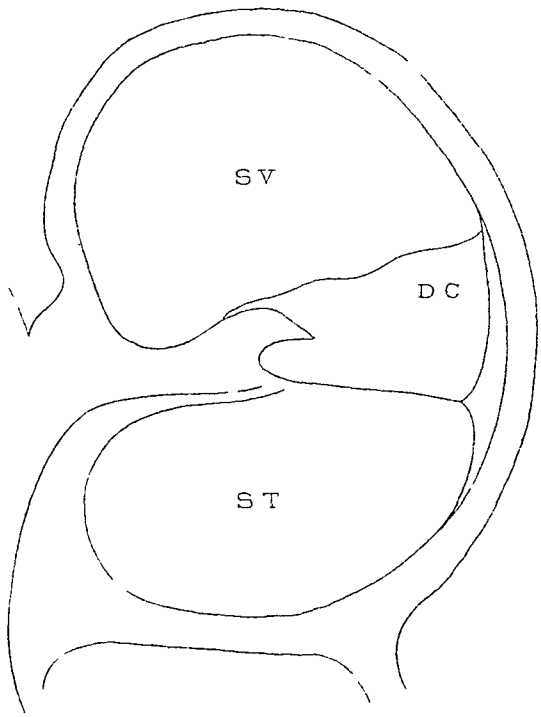

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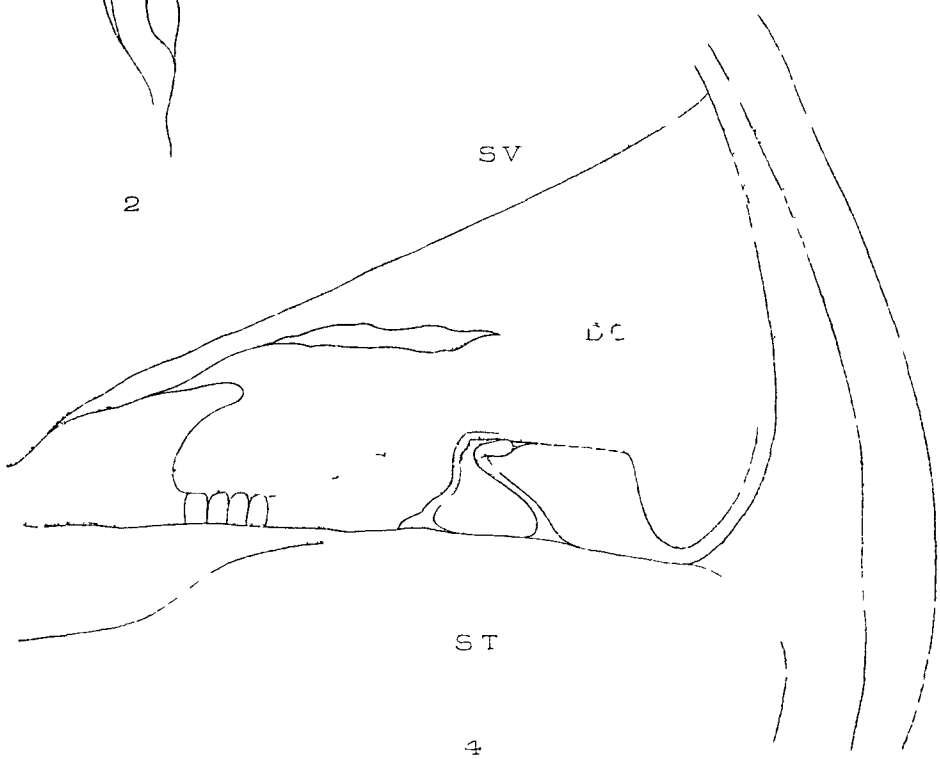

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membrane rests Corti's organ External and internal to it lie several rows of cells Beginning at the outer end of the basilar membrane observe the *supporting cells of Hensen*, and then a row of cells with fine bristles projecting from their upper surface—the *outer hair-cells* These cells occur in three or four rows Passing onwards we come to the *arch of Corti*, composed of an outer and an inner rod or pillar, each in a single row Each rod has the shape of an *f*, and they are so arranged as to touch with their upper part or head, whilst the base rests on the membrana basilaris, thus leaving a triangular space between them Each rod consists of a head, a narrow middle part or body, and a broad, wing-shaped base or foot The head of the inner rod has a concavity on its outer surface which receives the convex surface of the outer rod A flat process or *head-plate* proceeds outwards which helps to cover in the head of the outer rod It terminates in the membrana or lamina reticularis The outer rod also sends outwards a short process which forms the first phalanx of the membrana reticularis The outer rods are longer, thicker, and more numerous than the inner rods At the foot of each rod, on the surface directed towards the triangular tunnel under the rods, is a small nucleated mass of protoplasm

The *inner hair-cells* of Deiters lie immediately inside the inner rods They occur in a single row, and possess fine hairs, projecting from their free surface just like the outer hair-cells Internal to these there is a row of nucleated columnar epithelial cells, which support these cells They are continued inwards over the membranous portion of the membrana basilaris as far as the sulcus spiralis The *lamina* or *membrana reticularis* will be seen in section It is a cuticular, hyaline, very resistant membrane which stretches outwards from the heads of Corti's rods to the outer hair-cells In it exist a number of round or oval apertures, through which the outer hair-cells project The parts of this membrane which exist between these holes, being shaped like a finger, were called *phalanges* by Deiters (*Indicate the membrane of Reissner, Corti's rods and cells, and the membrana tectoria in* Pl XXVII, Fig 4)

The organs of taste (p 62) and touch (p 95) have already been alluded to.

THE MALE GENERATIVE ORGANS

THE TESTIS

PREPARATION (*a*) **Chromic acid and Spirit Mixture**—Use the testis of a dog, rabbit, or guinea-pig Make several cuts across the testis with a sharp razor, taking care to squeeze the organ as little as possible, and place it in the above fluid Change the fluid frequently, and at the end of ten days transfer it to weak, and then to strong, spirit Make transverse sections—by freezing—through the whole organ, stain them with logwood, and mount them in dammar They are apt to fall to pieces, but by floating the section in water all difficulty is overcome

(*b*) **Muller's fluid**—Harden the testis for three weeks in Muller's fluid, and then in spirit The testis is rendered very tough by this method, and it is less apt to fall to pieces

(*c*) **Interstitial Injection of Osmic Acid**—Inject into the testis, with a subcutaneous syringe, a one per cent solution of osmic acid, and harden the organ in alcohol This method shows the intertubular connective tissue very well after being stained with logwood

Transverse Section of the Testis **EXAMINATION** (L)—Observe the *capsule*, consisting of an outer layer—the *tunica adnata*, or the visceral layer of the tunica vaginalis—and an inner, dense, fibrous coat—the *tunica albuginea* Between these two layers oval openings are seen they are sections of lymphatic vessels From the capsule *septa* radiate inwards towards the corpus Highmori, so as to subdivide the organ into a series of compartments, each one of which is filled with small convoluted tubes—the *tubuli seminiferi*—which, of course, are cut in every possible direction These compartments, when filled with the tubules, constitute the *lobules* of the testis These septa have a decidedly lamellar structure, and they carry the large blood- and lymph-vessels Note these tubes of nearly uniform diameter, and observe the small amount of intertubular connective tissue, which takes the form of lamellæ of connective tissue covered with endothelial cells The interlamellar spaces communicate freely with each other, and form the origin of the lymphatics of the testis (p 118)

In some animals (dog, cat, and boar), columns of peculiar granular, nucleated cells are found between the tubules In some animals (guinea-pig) they are pigmented, but their exact nature is unknown

The *tubuli seminiferi* are relatively large, wavy, and convoluted tubes of uniform diameter, and are cut in every direction Each tube is seen to consist of a thick *membrana propria*, of a clear hyaline character, with flattened nuclei in it, so that it is probably endothelial in its nature It is more or less completely filled with seminal cells It may be possible to trace the tubules in their course towards the corpus Highmori, where they empty themselves into the *vasa*

recta, which in the corpus Highmori form the *rete testis* These tubes are much thinner than the seminal tubes, and there is a constriction where the one tube passes into the other They are lined by a single layer of low, columnar cells They lead into the wider *coni vasculosi* and the epididymis (p 117) (*Indicate the capsule and sections of the tubules in* Pl XXVIII, Fig 1)

(H) Study the **tubuli seminiferi**, especially in a transverse section Observe the thick membrana propria, within it are the *seminal cells*, arranged in several layers The *outer seminal cells*, next the membrana propria, consist of a single layer of transparent, polyhedral, nucleated, and faintly-defined cells Internal to these are *several* layers of cells—the *inner seminal cells* They are polyhedral, nucleated, somewhat granular cells, though they are usually more spherical in shape next the lumen of the tube

Study the innermost cells —some of them may be found with their nucleus partially divided, or even divided into two daughter nuclei, and then the whole cell divides, so as to give origin to two *daughter cells* These cells undergo further changes, and give origin to the spermatozoa, and are hence called *spermatoblasts* These cells, after certain intermediate changes in their nuclei and cell-contents, split up into a group of young spermatozoa, which are arranged in fan-shaped groups, the head or nucleus of each spermatozoon being directed towards the membrana propria of the tube This development of the spermatozoa in *groups* is quite characteristic, and several groups may be seen in a single tube, afterwards they become detached to constitute spermatozoa They are best seen in dammar preparations It is to be remembered that all these stages are not found in a section of any one tube, but several tubes must be examined to see all the stages (*Indicate the cells lining a tubule and the developing spermatozoa in* Pl XXVIII, Fig 2)

THE EPIDIDYMIS AND VAS DEFERENS

PREPARATION—Exactly the same as directed for the testis It is convenient to use the epididymis of a sheep Make transverse sections, stain them in logwood, and mount them in dammar, and do the same with the vas deferens

Transverse Section of the Epididymis **EXAMINATION** (L and H)—We traced the seminal tubules until they formed the rete testis This system of tubes opens into ten or twenty vasa efferentia, which form the *coni vasculosi*, which again open into the canal of the *epididymis*, and this again into the *vas deferens* Sections of the vasa efferentia and the convoluted tube of the epididymis are well seen, the latter being much larger than the former Each transverse section of the epididymis shows tubes with a relatively large lumen, lined with columnar epithelium, which rests on a membrana propria, supported by non-striped muscle The epithelium is columnar and ciliated, and the cilia are relatively long (*Indicate the general arrangement in* Pl XXVIII, Fig 3, *and the ciliated epithelium in* Fig 4)

At the bases of these cells branched pigment-cells are found, especially in the testis of the sheep Notice the normal amount of interstitial connective tissue, in which nerves and blood-vessels may be recognised

The **vas deferens** has a mucous, a muscular, and an outer coat, or adventitia The epithelium of this canal is columnar, and carries cilia only in that part next the epididymis

The **blood-vessels** of the testis are studied in the ordinary way

THE LYMPHATICS OF THE TESTIS

PREPARATION —Thrust the nozzle of a syringe into a perfectly fresh testicle, and inject into its substance a two per cent watery solution of Berlin-blue The lymphatics in the tunica albuginea and spermatic cord are rapidly filled Harden the testis in alcohol, and make sections, which are to be stained with picrocarmine and mounted in dammar, or in Farrant's solution The blue colouring matter passes into the origin of the lymphatics between the tubes, but none of it passes into them

EXAMINATION (L and H)—Observe the seminal tubules, stained of a yellowish-red colour, and between them note the distribution of the blue colouring matter, which has passed into the lymphatics

THE SPERMATOZOA

(A) IN THE LIVING CONDITION

1 **PREPARATION in the Newt**—Kill a large male newt (Triton cristatus), which may be readily recognised by the serrated crest along its back Remove the viscera, when the testes —two or three on each side—will be found as small round bodies, lying close to the spinal column Remove one of these, make a cut into it, and press out a little of its milky contents on to a slide, add a drop of salt solution , cover and examine

EXAMINATION (H)—Observe the seething mass of spermatozoa Each one consists of a so-called *head*, to which is attached a long, curved prolongation, the *tail* or *filament* Study one, and note that the tail moves with a rapid lashing motion, not unlike the action of a cilium, with the result of propelling onwards the spermatozoon

It is well to examine these spermatozoa with a higher power, with a lens of at least $\frac{1}{8}$-inch focal distance Each spermatozoon is then seen to consist of a *head*, and next to it is the *middle piece* of Schweigger-Seidel, and attached to this is the long, pointed *filament* or *tail* A long *spiral filament* is attached to this by a very transparent membrane (Leydig, H Gibbes)

2 **In a Mammal, *e g* a Sheep**—Make a cut into the globus major of a perfectly fresh testicle, and press out a little of the milky seminal fluid, and examine it as above

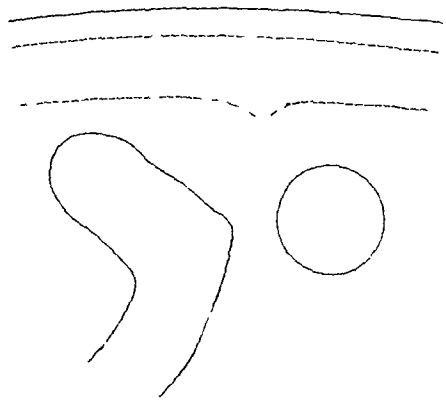
(B) PERMANENT PREPARATIONS

1 **Spermatozoa of the Newt**—Harden the testis of a newt in five per cent ammonium chromate for twenty-four hours , wash away all the colouring matter, and divide a testis, and then press out a little of the fluid, which is next mixed with a drop of glycerine, covered and preserved in the ordinary way The spermatozoa of a newt may be stained with logwood and an aniline dye, but the process is rather difficult, and requires much time and practice

2 **Mammalian Spermatozoa** (*a*)—Place a little glycerine in a watch-glass, and add to it a few drops of absolute alcohol Make a cut into the globus major of a perfectly fresh testis Press out some of the fluid into the watch-glass, and mix it thoroughly with the glycerine fluid Place a drop of this on a slide, and cover (Pl XXVIII , Fig 5)

(*b*) Another way is to place a drop of seminal fluid on a slide and allow it to dry, and then cover and seal it up *dry*, without the addition of any fluid

PLATE XXVIII TESTIS & OVARY



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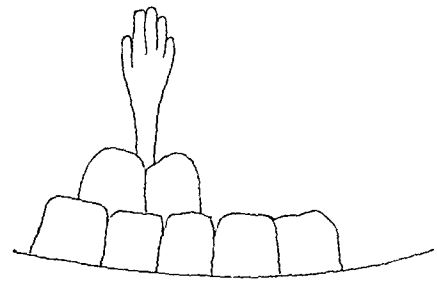

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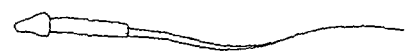

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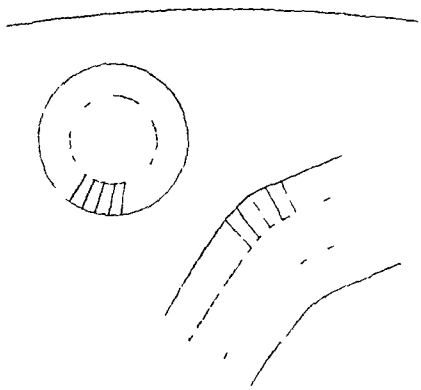

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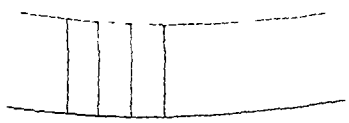

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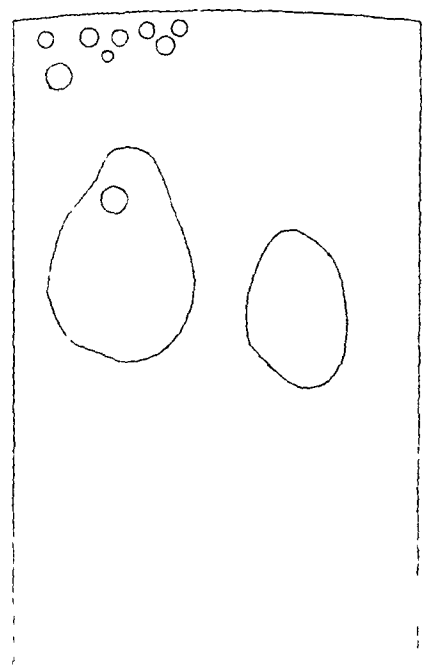

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THE PROSTATE GLAND AND PENIS

These are best hardened in the chromic acid and spirit mixture It is well to examine a section of a penis with its blood-vessels injected, to study the venous sinuses in the *corpus cavernosum penis* The glans penis, when stained with gold chloride reduced by the acetic acid method, shows the mode of termination of medullated nerve-fibres between the epithelial cells covering it, and also their mode of termination in *end-bulbs* In the prostate numerous sections of branched tubular glands lined with columnar epithelium, and supported by much non-striped muscle, are to be found

THE FEMALE GENERATIVE ORGANS.

THE OVARY

PREPARATION (*a*) **Chromic Acid and Spirit Mixture**—Harden the entire ovary of a cat, rabbit, or bitch in the above fluid for seven to ten days, and then transfer it to weak, and afterwards to strong, spirit Make transverse sections, stain some with logwood, and mount one in dammar, and another in Farrant's solution

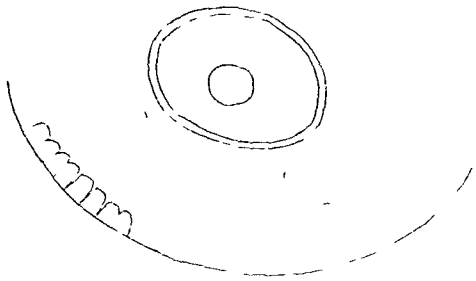
(*b*) **Muller's Fluid**—Harden the entire ovaries of any animal in Muller's fluid Change the fluid at the end of the first day, and continue the hardening for three weeks The specimens must on all occasions be handled very gently, to avoid the removal of the germinal epithelium covering them Transfer them to weak, and then to strong spirit Both methods yield excellent results

Transverse Section of the Ovary. EXAMINATION (L)—Observe the *framework*, which consists of (1) the tissue of the hilum, and (2) the stroma of the parenchyma The *tissue of the hilum* consists of loose areolar tissue, with many large blood-vessels The walls of the arteries are easily made out, because they are very thick The *stroma* consists of bundles of clear fusiform nucleated cells, with a small amount of fibrous tissue It is doubtful whether these fusiform cells are muscular or not Near the surface of the ovary are one or two layers of these cells, which usually contain no ova In the stroma small groups of polyhedral cells—*interstitial cells*—the rudiments of the Wolffian bodies, like those occurring in the stroma of the testis, may be found Note the single layer of short columnar epithelial cells—or *germinal epithelium* of Waldeyer—covering the surface of the ovary

Note the *Graafian follicles* lying in the stroma, and observe their distribution They are of various sizes and shapes, and the larger ones, *i e* the more developed, lie in the deeper part of the stroma, whilst the smaller ones form a layer two or three deep in the superficial part of the ovary (*Indicate the distribution of the follicles in* Pl XXVIII, Fig 6)

Study a large Graafian Follicle (L)—Observe the spindle-cells arranged more or less concentrically around it to form the *tunica fibrosa* Within this a *membrana propria*, lined with several layers of cells, which constitute the *membrana granulosa* of the follicle, and placed more or less excentrically is the *ovum* The ovum is imbedded in, and rests on, some of the cells of the membrana granulosa, and those cells on which it rests form the *cumulus proligerus*, and these cells are continued around the ovum, as the *tunica granulosa*, so as to embrace it A cavity, which is filled with an albuminous fluid, the *liquor folliculi*, exists between these two layers of the membrana granulosa The *ovum* is a complete cell, and consists externally of a hyaline cell-membrane, the *zona pellucida*, within this is the transparent yolk or *vitellus* Imbedded usually in one side of the yolk is the *germinal vesicle* (nucleus), with its included germinal *spot* (*Indicate the ovum and membrana granulosa in* Pl XXIX, Fig 1.)

PLATE XXIX OVARY, FALLOPIAN TUBE UTERUS



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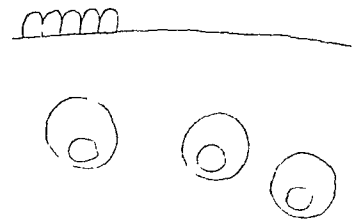

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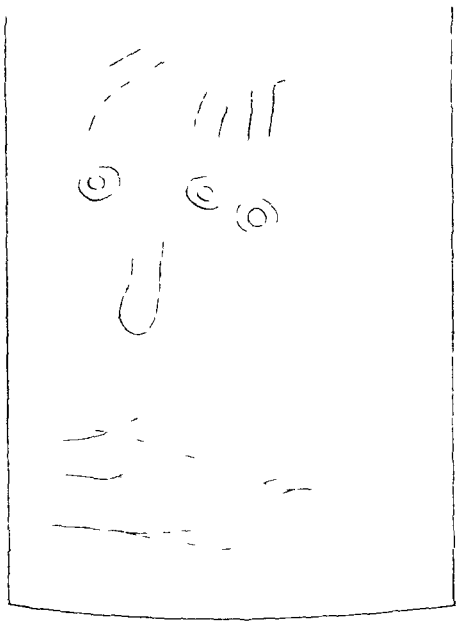

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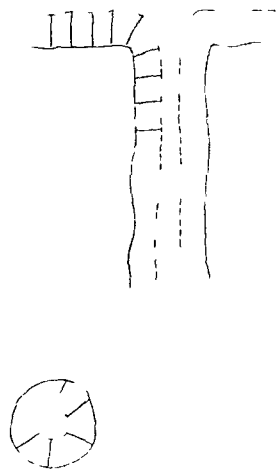

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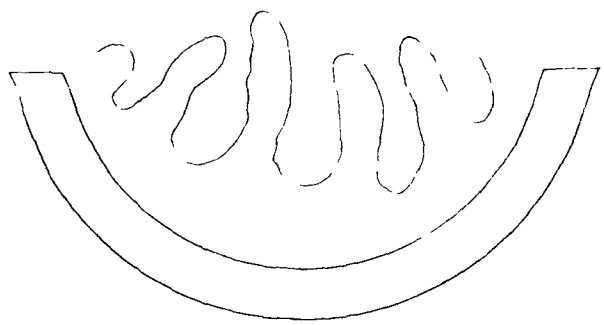

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(H) Study each of these parts from within outwards In the vitellus, yolk-granules and a fine reticulum of fibres may be detected

Study a Small, Unripe, Graafian Follicle (H)—These are found near the surface of the section Notice the ovum, with a relatively thin zona pellucida, and the membrana granulosa, consisting of, perhaps, only two layers of cells, and not yet separated into two distinct layers by the appearance of the liquor folliculi between them All gradations are found between these and a follicle ready to burst and discharge its ovum (*Indicate these, and the epithelium covering the ovary, in* Pl XXIX, Fig 2)

THE DEVELOPMENT OF GRAAFIAN FOLLICLES AND OVA

The ovaries of fœtal kittens or rabbits are prepared in the same way as directed for the adult ovary (p 120) Sections show the development of the ova from the germinal epithelium covering the ovary

CORPORA LUTEA

These are easily studied in the ovary of a cow, where they are very large Small pieces of one of them ought to be macerated in very dilute potassic bichromate, to isolate the large branched and pigmented cells which compose them Coloured crystals of hæmatoidin may be found Other pieces ought to be hardened in Muller's fluid for two weeks, and sections made to study the arrangement of the cells which compose them

THE FALLOPIAN TUBE

PREPARATION—Cut the Fallopian tube of a cat or bitch into pieces an inch long, and harden them for four days in the chromic acid and spirit mixture Make transverse sections, and stain them with logwood, and mount them in dammar

EXAMINATION (L and H) Observe the *serous*, or *outer coat*, composed of fibrous tissue, and within this the *muscular coat*, composed externally of a few muscular fibres, arranged longitudinally, and therefore cut transversely, whilst within these is a much thicker circular coat Then follows the *mucous coat*, consisting of fibrous tissue, containing many blood-vessels It is thrown into longitudinal folds by the contraction of the circular muscular fibres, and is lined by columnar ciliated epithelium It contains no glands Do not mistake the depressions between the folds of the mucous membrane for glands (*Indicate part of the wall of the tube in* Pl XXIX, Fig 5)

THE UTERUS

PREPARATION—Remove the uterus from a cat or bitch—preferably from an animal which has borne young—cut it in pieces an inch long, and harden them for five days in chromic acid and spirit mixture, and then transfer them to spirit Make transverse sections, and stain them with logwood, and mount them in dammar

EXAMINATION (L)—Observe (*a*) the *serous coat*, (*b*) the *muscular coat*, very thick, and composed of bundles of non-striped muscle, arranged in many layers The *mucous coat* consists of fibrous tissue, and is lined by columnar ciliated epithelium (the cilia are difficult to preserve), and in it are seen sections of the *uterine glands*, which are cut in every direction, but they are simple tubular glands, often branched at their lower extremities, and they are lined throughout by ciliated epithelium (Pl XXIX, Fig 3)

(H) Observe specially the uterine glands, with their membrana propria lined by ciliated columnar cells (*Indicate a gland in* Pl XXIX, Fig 4)

Similar preparations ought to be made of the cervix uteri, *e g*, of a cow

THE MAMMARY GLAND

PREPARATION—The best specimens are obtained from a gland taken from an animal near the full period of gestation Harden small pieces for a week in the chromic acid and spirit mixture, make sections, stain them with logwood, and mount them in dammar

EXAMINATION (L)—Observe the *framework*, which consists of septa, chiefly of lamellar connective tissue, which subdivide the gland into a series of small polygonal *lobules* In these septa sections of the large *lactiferous* or *milk ducts*, and large blood-vessels are found

Study a lobule (L and H)—Observe its shape, and note that it consists of a large number of *gland alveoli*, supported by a very small amount of connective tissue

Study an alveolus (H)—Observe its membrana propria, lined by a single layer of short columnar epithelial cells Sections of the large lobular ducts will be readily met with Fat-globules, *i e* milk-granules, may be seen within the protoplasm of these cells Osmic acid readily reveals the presence of fatty particles in the cells by blackening them (*Indicate the alveoli, with the cells lining them, in* Pl XXX, Fig 1)

THE MILK

Place a drop of fresh milk on a slide, apply a cover-glass and examine (H) It consists of a large number of oil-granules, of various sizes, floating in a fluid Each globule consists of an albuminous envelope (Ascherson's membrane), enclosing a globule of oil Add acetic acid, which partially dissolves the envelope, and then the oil-globules run into little heaps or clusters Osmic acid blackens them Do not preserve them (*Delineate in the upper half of* Pl XXX, Fig 2, *ordinary milk, and in the lower half the effect of acetic acid on it*)

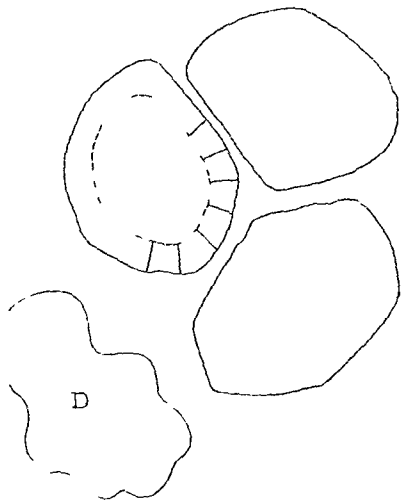
In the milk shortly after delivery many large nucleated cells, filled with milk-globules—the *colostrum corpuscles*—are to be found Examine these (H)

THE PLACENTA

PLACENTA OF A CAT OR GUINEA-PIG

PREPARATION—The animal ought to be killed about the middle of the period of gestation Open the abdomen and expose the uterus The uterus is cautiously opened,

PLATE XXX MAMMA, MILK PLACENTA



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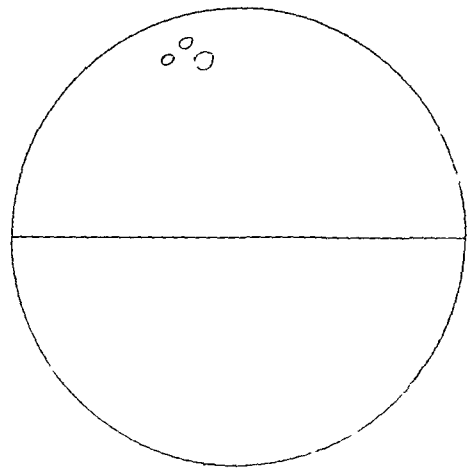

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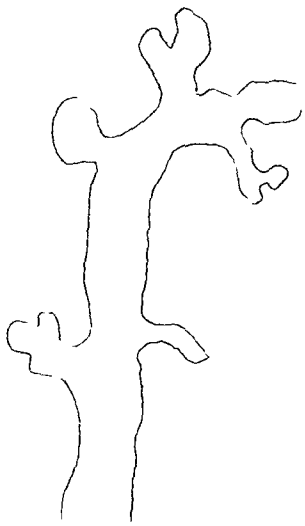

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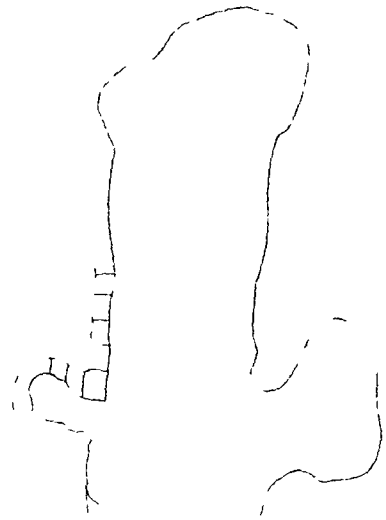

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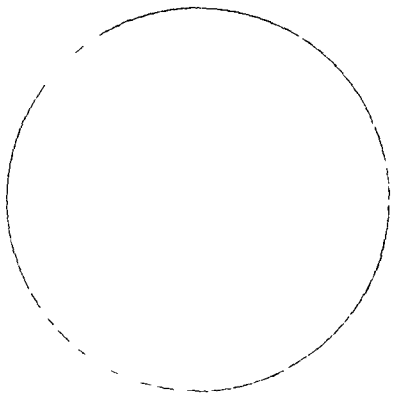

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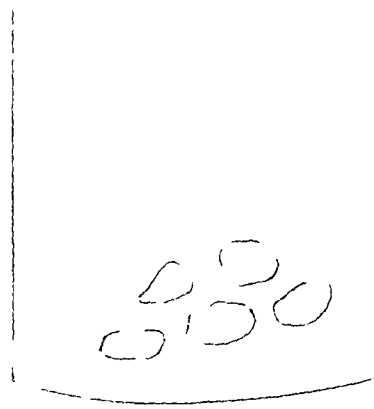

6

and care is taken not to sever the attachment of the placenta to the uterus Harden the uterus, placenta, and attached fœtus, which is still within the amniotic cavity, in the chromic acid and spirit mixture for two weeks, and then transfer them to dilute spirit, and afterwards to strong spirit The fœtus can be used for making sections of various organs Make vertical sections through the uterus and the placenta, and stain them with logwood or picrocarmine, and mount them in dammar or Farrant's solution

EXAMINATION (L)—Observe the uterus, with the placenta lying internal, and firmly attached to it The placenta consists of a narrow maternal part next the uterus, and a much thicker part—the fœtal portion In the uterus numerous sections of the enlarged uterine sinuses are seen The fœtal portion consists of richly branched villi, which are separated from each other by a relatively small amount of delicate connective tissue, and a layer of cells, which covers each villus Within these villi note the loops of blood-vessels Their distribution is easily seen, because they still contain the coloured blood-corpuscles

FRESH HUMAN PLACENTA

PREPARATION—Tease a small piece of a perfectly fresh placenta in salt solution

EXAMINATION (L)—Observe the large number of isolated and richly branched placental villi Each one contains a blood-vessel, whose course is easily seen, because it is still filled with blood-corpuscles A capillary loop is sent into each of the secondary branches on the villus The villus is covered by a layer of epithelium, whose nuclei are easily revealed by staining the section with magenta solution This epithelial covering varies in thickness on the same villus A delicate mucous tissue exists around the blood-vessels (*Indicate the shape and general characters of the villi in* Pl XXX, Fig 3)

(H) Observe the capillary blood-vessels filled with blood-corpuscles, and between the vessels note the corpuscles of the mucous tissue which supports them Study the epithelial covering It is low, columnar epithelium, with here and there masses of protoplasm, which contain many nuclei (*Indicate the epithelial covering and blood-vessels in* Pl XXX, Fig 4.)

HOW TO PRESERVE THE HUMAN PLACENTA

(1) **Osmic Acid**—A small piece of the fresh placenta may be teased in a one per cent solution of osmic acid, and mounted in glycerine

(2) **Dilute Alcohol**—Maceration for forty-eight hours in this fluid is an excellent means of isolating the villi They may be stained with picrocarmine

(3) **Muller's Fluid**—Harden very small pieces of a perfectly fresh placenta for three weeks in this fluid Make vertical sections, and treat them as directed for the placenta of a guinea-pig

THE FŒTAL MEMBRANES

THE DECIDUÆ

THE decidua vera and reflexa can be separated from each other near the placenta Remove a little of the tissue from the outer surface of the decidua reflexa, and examine it in salt solution It will be found to be nearly covered with fusiform cells, which are, for the most part, filled with fatty particles These cells may be stained with methyl-aniline, and preserved in a saturated solution of acetate of potash

The substance of the decidua consists of a tissue closely resembling mucous tissue If a small piece be treated with gold chloride (p xlv) branched connective-tissue corpuscles are obtained

THE AMNION

A small piece of this membrane, after being hardened for five days in Muller's fluid, is thoroughly washed, and placed on a slide, and stained with picrocarmine Observe on its inner surface a layer of somewhat flattened cells

THE UMBILICAL CORD

PREPARATION—Cut a fresh cord into pieces an inch in length, and harden them in Muller's fluid for two days, and afterwards in the chromic acid and spirit mixture for a week Make transverse sections Stain one with logwood, and mount it in dammar, and another with picrocarmine, and mount it in Farrant's solution

EXAMINATION (L)—Observe the circular shape of the cord, containing sections of three blood-vessels, *i e* two umbilical arteries and a larger umbilical vein These are surrounded by a modified mucous tissue (p *28*), called *Wharton's jelly*, and outside all is a layer of thin, nucleated, polygonal cells The tissue is denser around the blood-vessels In Wharton's jelly are found spaces—the sections of canals that run in the cord They are most obvious near the circumference Note the mucous corpuscles amongst the fibres (*Indicate the umbilical arteries and vein, and Wharton's jelly, in* Pl XXX, Fig 5)

(H) Examine the walls of the arteries and vein, and note their great thickness Study the branched corpuscles of Wharton's jelly (*Indicate the spaces and the mucous tissue in* Pl XXX, Fig 6)

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